

**A longitudinal study of the body composition
of children with cystic fibrosis compared to
healthy children using the reference four-
component model with an assessment of
clinical tools available for body composition
measurements**

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Statement of originality

I, Jane Williams confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature.....

Date.....

Abstract

Body composition (BC) is an important prognostic factor in patients with cystic fibrosis (CF). International guidelines recommend monitoring growth and nutritional status in children with CF using simple anthropometry. However, methodological issues with simple techniques are more significant in children due to growth and maturation, and even more problematic in patients, perhaps accounting for inconsistent findings in previous BC research in children with CF.

My thesis addressed three aims: 1) comparison of BC in young children with CF and controls using the criterion four-component model (4CM), cross-sectionally and longitudinally using pair-, group-match and reference database comparison ; 2) investigation of relationships between BC and lung function (FEV_1); and 3) evaluation of simpler BC techniques for clinical assessment of children with CF.

Results

1) Using the 4CM I found sex differences not identified by simpler techniques; girls with CF had abnormal baseline body composition, whilst longitudinal analysis showed deteriorating fat-free mass (FFM) in both sexes. Conclusions differed according to the comparison group used, perhaps accounting for some inconsistencies between previous studies.

2) Contrary to previous research, using the 4CM fat mass was positively associated with FEV_1 in girls; this association was not apparent at 2 year follow-up despite declining FEV_1 . FEV_1 was associated with FFM in boys and bone mass in girls, in accord with previous research.

3) Simple BC techniques were not interchangeable, and dual-energy X-ray absorptiometry (DXA) on its own or in combination with bio-electrical impedance (BIA) gave results closest to the criterion method.

Conclusion

Using the 4CM, abnormal BC and associations between BC and lung function were detected, which were not apparent using simple anthropometry. The findings emphasise the importance of using appropriate techniques to measure BC in children with CF, and suggest that DXA with or without BIA may be most appropriate in clinical practice.

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Abbreviations

2CM	two-component model
3CM	three-component model
4CM	four-component model
%IBW	percentage of ideal body weight
ADP	air-displacement plethmysography
BCM	body cell mass
BIA	bio-electrical impedance analysis
BMC	bone mineral content
BMD	bone mineral density
BMAD	bone mineral apparent density (size adjusted BMD)
BMI	body mass index
CF	cystic fibrosis
CT	computerised tomography
CFTR	cystic fibrosis transmembrane conductance regulator
Cl	chlorine
DV	dependent variable
DXA	dual-energy X-ray absorptiometry
FEV ₁ %	percentage of the expected forced expiratory lung volume in 1s
FEV ₁ SDS	forced expiratory lung volume in 1 sec standard deviation score
FFM	fat-free mass
FFMI	fat-free mass index
FM	fat mass
FMI	fat mass index
H _{FFM}	hydration of fat-free mass
IGF-1	insulin-like growth factor
LS	lumbar spine
MM	mineral mass

MMI	mineral mass index
MRI	magnetic resonance imaging
μSv	micro Sieverts
MUAC	mid-upper arm circumference
Na	sodium
P:M	protein to mineral ratio
PM	protein mass
PMI	protein mass index
R	resistance
REE	resting energy expenditure
SD	standard deviation
SDS	standard deviation (z) score
SE	standard error
SFT	skinfold thickness
TEE	total energy expenditure
TEM	technical error of the measurement
TBW	total body water
WM	water mass
Xc	reactance
Z	impedance

Chapter 1. Introduction

Cystic fibrosis (CF) is a lethal, autosomal recessive genetic disorder most prevalent in the white populations of Europe, North America and Australasia. A recent survey of the European CF registry notes 29,000 people affected in 35 countries, 16,500 of whom are children (1). The disease is characterized by malnutrition due to decreased absorption and increased nutritional loss and increased work of breathing due to chronic infection and inflammation. Consequently there is a negative energy balance which is associated with poor growth, reduced physical activity, lack of appetite and poor lung function. Improvement in diagnosing and treating children has increased life expectancy and consequently there is a growing need to address long-term as well as short-term health.

Assessment of growth and nutrition is an important part of the management of patients with CF, and there is increasing interest in measuring body composition because simple measures of weight and height cannot distinguish between predominantly metabolically active tissue (fat-free mass, FFM) and predominantly storage tissue (fat-mass, FM). Investigation of body composition in children with CF may be beneficial because of the potential to; (i) aid understanding of the mechanisms that underlie deficits in patients with CF, (ii) assess the effectiveness of medical and nutritional interventions and (iii) identify those children at most risk of deterioration. However, body composition measurements in children with CF are difficult because;

- Children are growing and maturation occurs at different rates, therefore, age and sex specific equations to calculate body composition must be used. However, these are often generated from healthy children and may not be appropriate for children with CF.
- Reference data on body composition in healthy children has, in the past, been limited. New reference data from our group, using a wide range of techniques,

allows assessment of patients' body composition by calculating standard deviation scores (SDS) (2).

- The readily available simple techniques used for measuring body composition either do not give any information about body composition (height, weight and body mass index (BMI)) or merely predict it from some other characteristic of the body (skinfold thickness; SFT, bio-electrical impedance; BIA).
- The most accurate body composition techniques are expensive, not readily available, are time consuming and require a high level of compliance from the child.

Much of the research and monitoring of the growth of children with CF have used simple measures of height and weight and crude measures of body composition such as SFT. In recent years there has been a growing use of more sophisticated and clinically available techniques such as dual-energy X-ray absorptiometry (DXA) in research studies but no studies have ever used a 'criterion' method of assessing body composition. The use of simple techniques or techniques that are biased in children/patients have led to inconsistent findings across research studies which have sometimes been confounded by large age ranges, no consideration of sexual dimorphism or no adjustment for short stature.

The aim of the work in this thesis is; (i) to investigate the effect of CF on the body composition of young children and whether this changes with growth and maturity, (ii) to relate components of body composition to lung function to identify whether baseline measurements or change over time predict later outcome and (iii) to compare the simple readily available body composition techniques to a criterion method in order to improve accuracy of body composition measurement in this group of children.

The organisation of the thesis is as follows: Chapter 2 covers the background to my research and a review of the previous research and the pertinent issues. Chapter 3 outlines the different techniques for measuring body composition and advantages and disadvantages of measuring children and patients with these techniques. Chapter 4

contains the hypotheses and the methods used to test them and Chapter 5 covers the pair-matched baseline measurements. The baseline and 2 year group-matched data (with reduced number) is presented in Chapter 6 and the data for longitudinal change in Chapter 7. Chapter 8 covers an assessment of clinical tools for measuring body composition compared to a criterion method in children with CF and Chapter 9 is an overall discussion and summary of the research with limitations of the study, recommendations for future research and clinical implications of the findings.

Chapter 2. Background

2.1 Cystic fibrosis

2.1.1 Incidence and aetiology of cystic fibrosis

Cystic fibrosis is the most common Caucasian lethal autosomal recessive genetic disorder, 1 in 25 people in the UK are carriers and 1/2500 live births are affected (3). A report of incidence in 35 European countries found there were 29,000 individuals with CF, 16,500 of whom were children (1). CF is caused by a mutation on the long arm of chromosome 7 and results in an abnormal functioning protein in epithelial cells, the CF trans-membrane conductance regulator (CFTR). The gene responsible is present in all cells but only expressed in certain cells: airway, sweat gland, pancreatic duct, vas deferens, bile duct and bowel epithelium and also the kidney and ependymal lining of the brain ventricles.

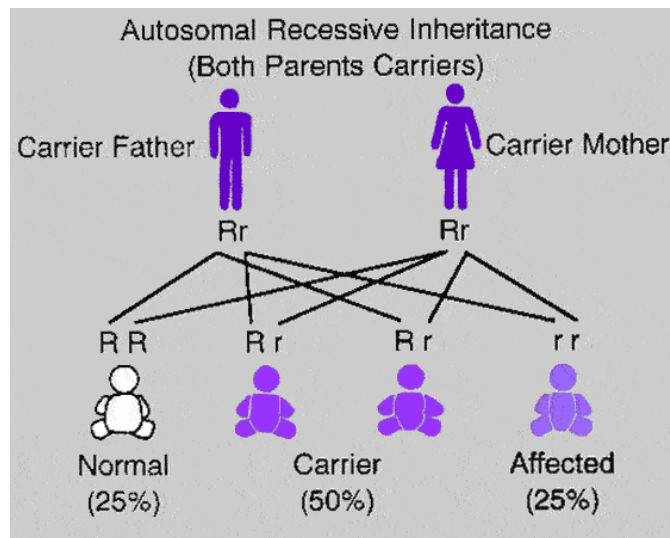


Figure 2.1. Inheritance pattern in cystic fibrosis

Over 1000 different mutations have been identified world-wide but in the UK 58% of people with CF are homozygous for $\Delta F508$ and 36% have 1 copy of $\Delta F508$ (4). The $\Delta F508$ mutation is associated with more severe clinical manifestations (5;6). There is a wide range of clinical presentation and severity with most presenting in early childhood with persistent respiratory tract infections and failure to thrive and around 15% of infants presenting with meconium ileus.

2.1.2 Symptoms

Severity of disease is not just related to genotype but is a reflection of a combination of factors including environment, adherence to therapy and genetic background. The CFTR protein regulates the movement of chloride (Cl) and sodium (Na) ions across epithelial surfaces and an inability to re-absorb Cl and Na ions from the sweat gland ducts leads to a high salt content of sweat and is therefore used as a diagnostic test. The major dysfunction of interest to the work presented in this thesis is exhibited in;

1) The lung; although the mechanism is not completely understood it is likely that an inability to secrete Cl and excess re-absorption of Na ions in the lung epithelia may lead to poor hydration of the airway mucus. This leads to thick sticky mucus which impairs ciliary clearance and results in plugging and bacterial infection (typically *Pseudomonas aeruginosa* and *Staphylococcus aureus*), in turn inducing a damaging inflammatory response and irreversible lung damage. Lung disease has the greatest impact on morbidity and mortality (7) and there is a specific decline in lung function over time dependent on initial lung function and gender (8). Sex differences in survival of young people with severe CF related lung disease have been noted by Aurora et al (9) who calculated survival odds were lower in girls considering several factors in children referred for lung transplantation. The inter-relationship between lung function and nutritional status has been well documented but the direction of the relationship is unclear (10;11).

2)The pancreas; dehydrated, protein-rich secretions block the proximal ducts and digestive enzymes are retained in the pancreatic ducts causing a loss of functioning tissue which is replaced by fibrosis and fatty tissue. Pancreatic insufficiency occurs in 85% of cases with consequent malabsorption of fat, protein and fat soluble vitamins leading to steatorrhea and malnutrition. Malabsorption of carbohydrates is minimal (12). The prevalence of malnutrition defined by weight for height has been reported as 19% of 2-6 year German children in 2000 (13), 23% of those children on the US registry in 2005 (14) based on weight and height <90% expected and data from the UK CF registry revealed that the mean weight SDS of the males were between -0.25 and -0.5 until the age of 10 years after which time both weight and BMI SDS declined. The mean weight SDS of females was stable over time at -0.5 but they had a declining BMI after the age of 5 years (15).

The combination of lung pathology and malabsorption inevitably impacts on overall energy balance. Approximately 60-70% of total energy expenditure (TEE) is accounted for by resting energy expenditure (REE) and studies of patients with CF suggest that there is an increase of 7-35% (16-18).

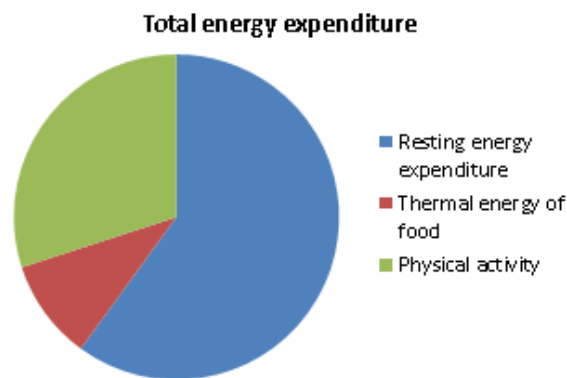


Figure 2.2. *Components of total energy expenditure*

Magoffin et al (18) studied children with CF and found that REE was elevated compared to controls and that this was higher in the girls with CF ($109\% \pm 9.5\%$ of predicted) compared to boys with CF ($103\% \pm 7.0\%$ of predicted) and the difference was apparent throughout puberty. This sex difference is supported by a study by Allen et al (19). In a study of 19 infants with CF compared to controls Bronstein et al (20) found that increased REE expenditure may not be associated with increased TEE if differences in body composition are accounted for. In this study they found that energy expenditure was greater in children with CF compared to healthy controls if calculated on the basis of weight using kilocalories per kilogram per day but not if based on FFM using kilocalories per kilogram of FFM per day. Another explanation for no increase in TEE in children with CF may be that increased REE is compensated for by reduced physical activity (16;20).

REE may be raised due to pulmonary infection and inflammation (21;22) although a more recent study reported that pancreatic status is the most influential factor for increased REE (23) and another suggests that there is no association of REE with lung function or liver disease either cross-sectionally or longitudinally (18).

In summary, typical symptoms for an untreated person with CF are; recurrent respiratory infections, coughing, failure to thrive, loose stools and male infertility. In addition complications including sinusitis, pancreatitis, nasal polyps, liver disease, diabetes, gallstones, oesophageal reflux, and low bone mineral density and energy imbalance are possible.

2.1.3 Overview of treatment

At birth the lungs are normal and therefore the primary goal of management is to maintain good lung function by the prevention of infection, reduction of mucus viscosity and removal of secretions. A regimen of regular chest physiotherapy or airway clearance is usually instituted, inhaled medication and antibiotics are usual. For patients with chronic infections it may additionally be necessary for oral antibiotics, inhaled mucolytics and in

some, intra-venous antibiotics. Birth weight is not different from that of healthy children (24-26) but weight gain is lower if a diagnosis is not made in the neonatal period (27;28) and one study found that those children diagnosed and treated from birth were heavier, longer, with larger head circumference after 13 years than those in whom diagnosis was delayed (28). Another study of infants with CF at birth found lower length SDS (-1.24), weight SDS (-0.72) and head circumference SDS (-1.82) (29) although a third of the subjects were affected by meconium ileus which is associated with worse outcomes. After 4 years length and weight SDS had improved although head circumference SDS remained 1 SDS below the mean. Pancreatic damage may occur before birth (30) and dietetic management is important because of the recognised relationship between optimal nutritional status and improved lung function, reduced medical intervention and improved mortality. Whereas malnutrition was once an inevitable outcome, improved treatment and nutritional support has reduced the prevalence (31).

Dietary management involves an increased energy intake of between 120-140% of the usual recommended intake to counteract the detrimental effects of the increased respiratory effort, chronic infection and inflammation, decreased absorption and increased nutritional loss. To achieve this increased energy intake, some patients may require oral supplements or enteral nutrition, for example by gastrostomy feeding. Patients with pancreatic insufficiency take oral pancreatic enzyme supplements to assist in nutrient absorption although this may not completely correct malabsorption (32). In addition, fat soluble vitamins are given and calcium supplements may be required.

A study by White et al. (33) found that 58 children with CF aged 5 – 16 years had wide ranging energy intakes of between 44-163% of the recommended amount and that despite a higher energy intake per kg of body weight than controls they were unable to achieve optimal growth. Appetite and energy intake may be deleteriously affected by gastro-oesophageal reflux, intestinal obstruction, inflammatory response to infection and CF related diabetes. In addition, psycho-social factors may impact on appetite and energy intake.

Delay in onset of puberty and menarche has been reported in patients with CF and nutritional problems (34;35) and also in well-nourished females (35). However, other studies have found that puberty is not significantly delayed in girls with CF compared to controls (36). As the child grows other issues such as low bone density, gastro-intestinal and liver complications and CF related diabetes may need to be addressed and lung transplantation for those with serious lung disease is an option. Survival after transplantation has been reported as 82% after one year, 70% at three years, 62% at five years and 51% after 10 years (37). For adults with CF, fertility and pregnancy introduce more challenges to clinical management. For both children and adults psycho-social factors may impact on the efficacy of treatment regimens.

2.1.4 Prognosis

Early diagnosis, improved treatment, lung transplantation and overall better understanding of the disease process have increased life expectancy considerably; the median estimated life expectancy of children born in 1990 is 40 years (38). However, several long term complications such as osteoporosis have become apparent in young adults with CF and it has been recognised that it is important to optimise nutrition in childhood to improve quality of life and prognosis (39).

2.2 Body Composition changes during normal growth

Sexual dimorphism in body composition is evident from foetus to adult but becomes most apparent during puberty. Wells' (40) comprehensive paper on sexual dimorphism of body composition explains why, in humans, increased energy needed for lactation which provides energy for the growth of a large brain in the offspring, has favoured smaller, fatter females compared to males. At birth, the sexes have similar FM but boys are longer and have more FFM adjusted for height than girls. This can be seen in the following Hattori chart of FM index (FMI: $\text{FM}/\text{height}^2$) plotted against FFM index ($\text{FFM}/\text{height}^2$) from birth to 1 year.

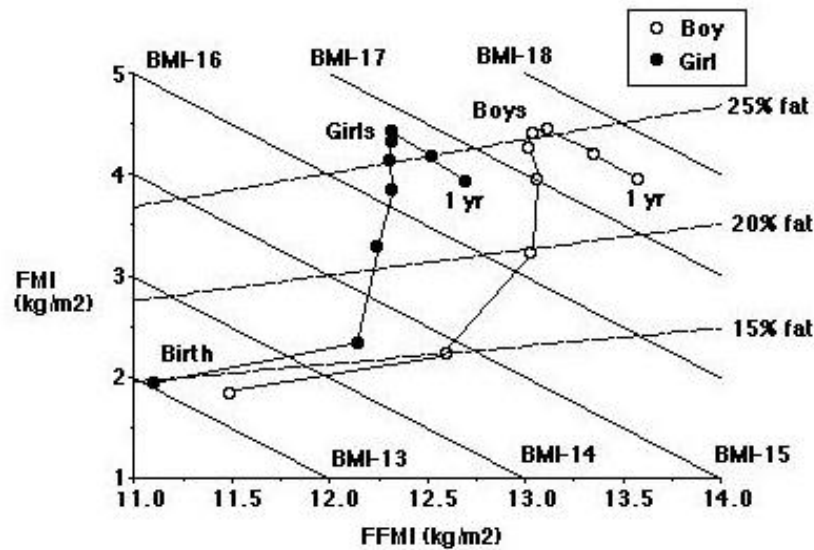


Figure 2.3. Hattori graph showing change in fat mass adjusted for height (fat mass index; FMI) v fat-free mass adjusted for height (fat-free mass index; FFMI) in children from birth to 1 year. (41)

(Permission to reproduce this has been granted by Professor JCK Wells).

The following 2 graphs from age 1 to 10 years and 10.5 – 18 years demonstrate how the differences remain throughout childhood and adolescence.

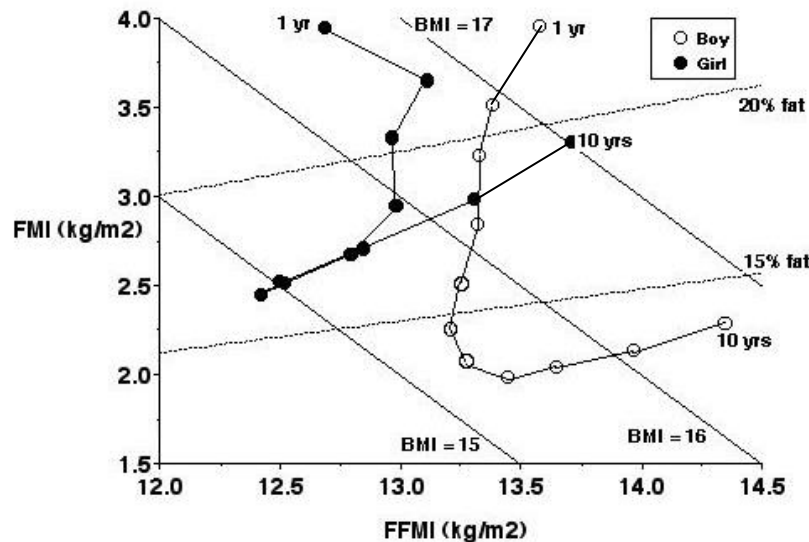


Figure 2.4. Hattori graph showing change in fat mass adjusted for height (fat mass index; FMI) v fat-free mass adjusted for height (fat-free mass index; FFMI) in children from 1 year to 10 years (41)

(Permission to reproduce this has been granted by Professor JCK Wells).

These graphs adjust for the difference in height between the sexes and the males show greater overall FFM (including mineral mass; MM) and lower FM after age 5 than females. However, females enter puberty earlier, have more rapid pubertal development whereas boys have a longer growth period (42). Bone mineral content (BMC) is similar in the sexes till age 16 years (43) but is significantly different after that age (44;45), males having greater BMC particularly in the appendicular skeleton. Shape and regional tissue distribution also differs so that ratios such as waist to hip and SFT measures need to be compared within a sex.

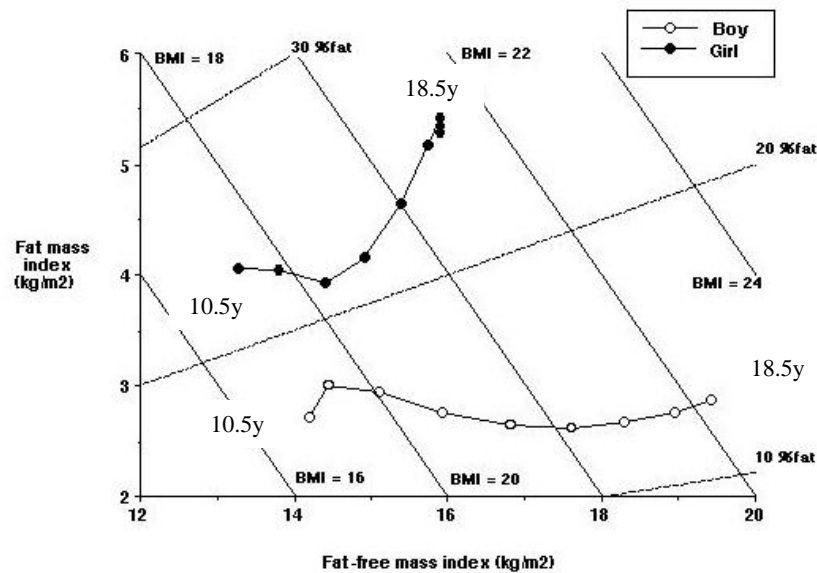


Figure 2.5. Hattori graph showing change in fat mass adjusted for height (fat mass index; FMI) v fat-free mass adjusted for height (fat-free mass index; FFMI) in children from 10.5 to 18.5 years(41).

(Permission to reproduce this has been granted by Professor JCK Wells).

2.3 The impact of nutrition on body composition

Childhood and adolescence are periods of rapid growth and development requiring a diet providing sufficient quantities of the 3 major food groups; fats, carbohydrates and proteins and minerals and vitamins. Body composition is influenced by many factors; genetic, hormonal, activity level and state of health but a major factor is that of nutritional intake. There is little research on the direct impact of nutritional intake on body composition in children with more likely outcomes of weight, height and BMI. Monitoring child growth by measuring height and weight gives an indication of under- and over-nutrition and wasting and stunting may be the first sign of a problem.

2.3.1 Under-nutrition

Under-nutrition leads to weight loss or failure to increase weight in the growing child due to an imbalance between nutrient intake and requirements. Chronic under-nutrition in children may lead to stunting. The imbalance may be due to reduced intake and/or absorption or increased requirements such as during sepsis, trauma, cancer or hypermetabolic states (46) or a combination of both. Where there is a deficit between intake and nutrient needs then body stores are used first to maintain the brain, since injury is rapid in the absence of glucose or ketones and second to maintain the structural elements of the body, cells and extracellular fluid containing protein. Energy is stored in the body as carbohydrates, protein and fat. Carbohydrate stored in the form of glycogen is only sufficient to provide energy for 24 hours and although body protein is sufficient to provide for the energy requirements for 30 days the structure of the organs would be lost. Fat is an expendable source of energy although it is not able to supply essential glucose for the brain. During periods of inadequate nutrition glucose for the brain is obtained by gluconeogenesis from protein catabolism and glycerol from lipolysis. If the energy deficit is prolonged fatty acids being transported to the liver are converted ketones which the brain can utilise and thus protein is spared. A study by Keys et al (47) examined the effect of 6 months of famine on a group of conscientious objectors in 1943 and showed that volunteers on this low calorie, low protein diet lost on average 17% of their lean mass and a marked fat loss of 73%. These volunteers returned to normal body composition after 1 year on a normal diet but malnutrition in patients may not be so easily corrected.

In patient groups, malnutrition may not only be due to inadequate intake or absorption but may be affected by abnormal hormone and cytokine profiles as a consequence of sepsis or trauma resulting in an increased metabolic rate or an abnormal metabolism due to disease. A study of patients with CF from 1 month to 17 years found that, although 12 infants diagnosed by neonatal screening had low body cell mass that was corrected by 1 year with therapy, children aged 2-17 years showed an increasing tendency with age for inadequate accretion of body cell mass (48). This is despite early

diagnosis and treatment. The same group report catch-up growth (weight, length and body cell mass) in 25 neonates with CF receiving conventional pancreatic enzyme and dietary therapy (24). In this group however, the mean FM remained significantly lower than expected at 1 year. Shepherd (49) in her review of nutrition in CF identifies the major consequences of malnutrition in CF as growth retardation, delayed puberty and specific deficiencies of protein, fatty acids, vitamins and minerals. If left untreated failure to thrive, growth failure, wasting and gross motor delay are likely. If the infant is fed a reduced useable protein source such as soya or has a low intake of protein, as in unsupplemented breast milk, secondary hypoproteinaemia with generalised oedema have been reported. Therapeutic regimes aim to correct the deficiencies. In a study of 10 growth retarded children with CF given nocturnal feeds of 120-140% of normal requirement for protein and energy for 1 year it was found that all had catch-up weight by 6 months and 9 catch-up height. Catabolism was reduced and there was a reversal in the trend for deteriorating lung function (50).

2.3.2 Over-nutrition

A positive imbalance between intake and expenditure over time will result in the excess energy being stored. In adults the consequent weight in relation to height (BMI) is used as a screening tool for overweight (greater or equal to 25) and obesity (greater or equal to 30). In adults BMI has a U shaped relationship with mortality. In children FM, FFM and height are changing rapidly, at different rates and particularly around the period of puberty where the timing may be affected by the amount of FM itself and therefore BMI cannot be used to classify overweight and obesity. In the paediatric setting BMI adjusted for age and sex, as centiles or SDS are used. However, even BMI centiles or SDS cannot distinguish between high FM and low FFM and vice versa for a given weight. Where nutritional therapy has been based on the weight of a child any weight gain may mask low FFM, in a study of patients with cerebral palsy given gastrostomy feeds it was reported that the gain in weight was due to a high FM (51). The universally recommended dietary intake for a child with CF is 120-140% of that of a healthy child.

Without measurements of actual body composition rather than just weight and BMI it is possible that the targeted weight gain may be excess FM rather than FFM and there is mounting evidence of obesity in children with CF (52). It is likely that if excess FM in a child with CF continues into adolescence and adulthood then the risk of complications such as those associated with cardio-vascular disease will be increased.

The effect of habitual diet on body composition is complicated particularly in children and patients where energy requirements may vary according to sex, age, puberty, activity and disease process. An accurate record of dietary intake over time is difficult, particularly in this group of children where appetite can change frequently. An experimental intervention with body composition as the outcome is the best way to investigate this relationship. This thesis does not take account of dietary intake although it is acknowledged that diet will impact on body composition.

2.4 Previous studies investigating the effect of cystic fibrosis on growth and body composition

A full explanation of the advantages and disadvantages of different body composition techniques is presented in Chapter 3. I will present here the findings of previous studies according to the techniques used with an explanation of the relevance of the technique in this particular group of patients. A summary of the most pertinent studies is presented in **Table 2.1**.

2.4.1 Anthropometry

Measurements of height, weight, circumferences and SFT have been used for many years for the assessment of growth and body composition. They are not recommended for evaluating body composition clinically in individuals or for short term changes in FM (53).

Indices of height and weight have been, and still are used to assess nutritional status in children by plotting on centile charts or by the calculation of percentage weight for age, percentage height for age, percentage weight for height and percentage of ideal body weight (%IBW). In 2001 in the UK the Royal College of Paediatrics and Child Health advised the use of BMI centile charts in preference to percentage weight for height and in 2002 the UK CF Trust recommended the use of software that uses UK 1990 growth data to generate SDS for height, weight and BMI (54). A study by Wiedemann et al (55) found that 4577 children with CF were reasonably nourished by %IBW but when using BMI centiles they were below the reference median. Another study (56) reported that %IBW underestimated malnutrition in children with short stature and overestimated it in those who are tall. In the United States the CF Foundation recommended the use of BMI centiles for the assessment of children and adolescents in 2008 (14). However, BMI cannot distinguish between FM and FFM as evidenced from the following graphs showing the body composition of 2 children measured for this thesis.

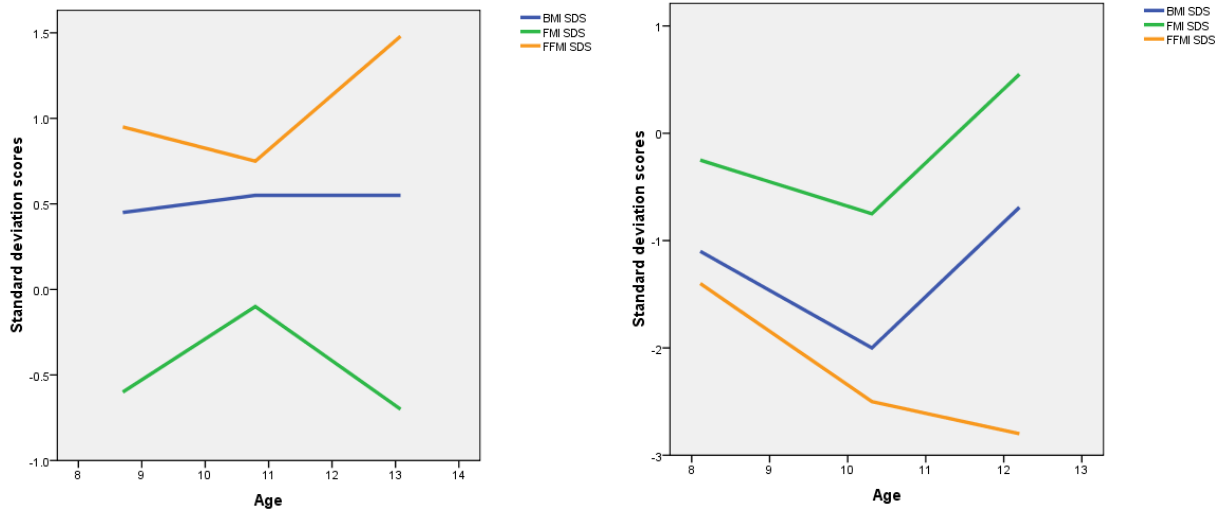


Figure 2.6. Graphs of body mass index (BMI; $\text{weight}/\text{height}^2$) indicated in blue, fat mass index (FMI; $\text{fat mass}/\text{height}^2$) in green and lean mass index (FFMI; $\text{fat-free mass}/\text{height}^2$) in orange, standard deviations scores (SDS) for two children with cystic fibrosis.

The first graph illustrates that this child has a higher FFM and lower FM SDS compared to BMI SDS and the second graph depicts a child with high FM and low FFM compared to BMI SDS.

Stunting (57) and wasting (58) have been shown to be independent predictors of survival and the lack of short stature amongst adults may be due to survival of well nourished patients rather than improved growth in the undernourished (59). Low weight for height in adults occurs particularly in females and is correlated with more severe lung disease and reduced survival (60;61). McNaughton et al (62) comparing indices of height and weight with nutritional status assessed by total body potassium counting found that height and weight were not sensitive indicators of suboptimal nutritional status defined by total body potassium SDS of ≤ -2 or $< 80\%$ of predicted in 226 children with CF. The literature is consistent in finding that children with CF tend to be shorter and lighter than their healthy counterparts (15;55;63-65) and a recent study in Poland (66) noted that the children were disproportionately shorter in the legs compared to the trunk.

Longitudinal studies suggest that weight for age and height for age decline over time, are associated with declining lung function and that there are differences between the sexes (10;13;67;68). However, despite CF being a disease of malabsorption there are now reports of obesity. Kastner-Cole (52) reviewed the records of 3,000 patients, from infant to adult, on the UK CF registry and found the incidence of obesity to be 1.4% in boys and 1% in girls under 18 years and 1.6% in men and 0.2% in women. My own data of BMI SDS in young children with CF (69) found an obesity level of 10.8% in boys and 0.02% in girls. However, due to the inability of BMI to distinguish between FM and FFM the proportion with a high BMI due to elevated FM (assessed by the 4-component model; 4CM) was only 8% of boys. In 'healthy' populations high BMI has a negative relationship with forced expiratory volume in 1 second percentage of expected ($FEV_1\%$) in children (70) and adults (71) although in the CF population a positive relationship has been demonstrated (11;72) and Forrester et al (73) found that high BMI SDS independent of muscle mass in 2096 clinically stable adults with CF was associated with better FEV_1 . This study used serum creatinine as a marker of total skeletal muscle and the authors acknowledged the need for

caution when using this marker in those with extremes of muscle mass. However, the fact that BMI is positively associated with FEV₁ regardless of muscle mass is interesting and the finding that those with BMI ≥ 25 kg/m² have the highest values for FEV₁ may be a reflection of their better clinical status compared to those with lower BMI. As previously discussed BMI does not distinguish between FM and FFM and the discordance between the negative relationship between BMI and spirometry in healthy subjects and the positive relationship in adults with CF may suggest that a given BMI represents different body composition in these two groups.

SFT at 3 or 4 sites is often used as a proxy for overall body fat. Some studies inappropriately quantify FFM using SFT (74;75) (since measuring sub-cutaneous fat does not give information regarding FFM), whilst other studies introduce error by converting raw SFT data to % fat using published equations that may not be relevant to children with CF (76). Indeed, in my own study comparing SFT converted to FM by 2 different prediction equations, I found that the bias varied between 3 and 21% of the mean value depending on equation, sex and whether the child was healthy or had CF (69). Arm anthropometry (mid-upper arm circumference; MUAC and tricep SFT) plotted on centiles from reference data (77) has been recommended in a US consensus document on the management of CF (78) to monitor FM and FFM clinically. However, a study by Chomtho et al (79) comparing arm anthropometry to the criterion 4CM and DXA in 110 healthy children and 40 children with CF found the measurements predicted FM but not FFM.

2.4.2 Bio-electrical impedance

BIA is a prediction technique, that is to say TBW is predicted by resistance to a flow of electricity through the body rather than actually measuring FFM. The accuracy of prediction techniques such as BIA is assessed by comparing to a robust reference method (one that is not a prediction technique). Thereafter, using regression analysis a population specific prediction equation can then be generated for use in a similar population. Suitable reference techniques actually measure FFM or a proxy of FFM such as water or body

potassium and include the 4CM, total potassium counting, DXA and hydrometry (described in Chapter 3). Prediction equations are often generated in healthy populations which may not be suitable for patient groups and few equations have been derived for patients with CF.

Previous body composition studies have concluded that BIA predicts total body water (TBW) (80;81) or total body potassium (82) in children and adolescents with CF and may be used with equations derived from CF populations to predict FFM. Other studies have used inappropriate reference methods for comparison with BIA such as SFT and MUAC and therefore the conclusions can be questioned (83;84). A study of adults with CF by King et al (75) compared FFM predicted from 2 BIA equations based on healthy adults (85;86) compared to DXA and found that although FFM by each technique was correlated, one equation overestimated FFM in women and the other underestimated FFM in men with CF. Eisenmann et al (87) studied 3-8 year old children and compared BIA to DXA concluding that BIA has 'limited utility in estimating body composition'. However, although several studies have generated prediction equations for FFM in a paediatric population by the incorporation of the relationship between $\text{height}^2/\text{impedance}$ and factors such as age, sex and weight (88-91), there is limited research specifically in children with CF. A literature search revealed only one study that generated a prediction equation for TBW in CF children and adolescents by comparing to deuterium dilution (92). Prediction of TBW and thereby FFM may be affected deleteriously in the CF population by disturbances in skin electrolyte composition, abnormal fluid distribution, sodium depletion and hydration level (80;93;94). Using BIA a previous study found the greatest decline in nutritional status occurs after the onset of puberty, particularly in girls (59) although other studies using DXA, suggest that deterioration in growth and nutritional status occurs throughout childhood (36;95).

2.4.3 Hydrometry

Some studies have utilised hydrometry, often in conjunction with other techniques to investigate body composition of children with CF. Where hydration of the FFM is within normal range this technique is one of the more accurate ways to calculate FFM. I am not

aware of any studies of children with CF that have noted abnormal hydration of the FFM. Stettler (96) noted a reduction in FFM in boys only and another study found both boys and girls had less FM and FFM than controls (74) using hydrometry and SFT. Not all studies identify deficits of body composition in children with CF; Marin et al (97) using anthropometry and hydrometry found no difference between 15 children with CF and controls, however, not all subjects were matched with same sex controls.

2.4.4 Dual-energy X-ray absorptiometry

Low bone mineral density (BMD) has been reported in both children (64) and adults (98) although other studies report normal BMD in young children (99;100). There has been extensive research into the relationship between bone and clinical outcome in CF but for the purposes of this thesis I will be concentrating on the relationship between soft tissues and clinical outcome. Soft tissue assessment by DXA has become more widely used due to its increased availability in recent years. There are inherent problems for soft tissue assessment because of the assumptions made in the software and a large study of healthy children by Shypailo et al (101) concluded that it could not be considered a reference method. In my own study comparing DXA to the criterion 4CM in healthy children and those with a variety of diseases I found that bias was affected by size, sex and whether the subject was healthy, obese or had CF or glycogen storage disease (102). DXA significantly underestimated FFM in girls with CF but not boys although the age range was narrow (8-11 years) and the numbers small (n=26). However, despite its limitations DXA is the most sophisticated of the body composition techniques likely to be available in a clinical setting. There is a paucity of research into the best way to utilise the data from DXA in this group of patients; whole body and regional bone and soft tissue data is generated but there is a lack of reference data in children and a lack of research as to whether the components of body composition should be size adjusted when compared to a reference group.

Several recent studies of children and young people with CF using DXA have noted a reduction of FM, FFM and BMD (36;65;103). Depletion of these tissues becomes more severe with increasing age (36;67). Even with normal BMI, FFM and BMD depletion has

been noted in adults (75;104) and in one study of children and adolescents 54% were found to have a lean mass of ≤ -2 SDS with % IBW of $\geq 85\%$ (105) although size adjustment in this study may not have been ideal. This is referred to as ‘hidden depletion of FFM’ because monitoring using BMI would not reveal a problem.

2.4.5 Total body potassium

Body cell mass (BCM) is the metabolically active part of FFM and therefore if accrual during growth is suboptimal nutritional status will be affected. 98% of 40K is in BCM so the most common way to measure BCM is with a total body potassium counter. These are quite rare and the research using this technique in patients with CF is predominantly from Toronto where several studies have investigated the effects of different feeding regimens (106-108) or from Brisbane where the research question often addresses the relationship between BCM and growth and nutritional status and, more recently, REE (24;48). Because this technique does not measure absolute values of FM and FFM, direct comparison with other body composition techniques is difficult. The technique is hampered by the fact that conversion of total body potassium to BCM uses an equation derived in healthy adults (109) and in addition there is sparse reference data that allows for adjustment for height and sex although the Brisbane group have generated their own.

Shepherd et al (89) evaluated total body potassium in 140 patients with CF and found suboptimal growth was associated with deficits in growth of BCM. Thomson et al (95) found that in 61 children with CF, those with normal BCM had significantly less decline in spirometry over 2.4 years and they recommended the use of the technique to predict those children at risk of greatest decline. Murphy et al (110) assessed BCM in a group of 64 children aged 10.6 ± 2.9 with CF and using their own reference data found that the children had a mean BCM SDS of 0.54 with boys slightly higher than girls but with more variability between individuals. Only 2 children were considered to have suboptimal nutritional status. Neonatal screening for CF has been common place in Australia for several years, unlike the UK and this may account for the good nutritional status of these children.

2.4.6 Longitudinal studies of body composition in cystic fibrosis

One of the problems of interpreting data from published studies is the different age ranges, particularly in the growing, maturing child, and particularly with the changes associated with pubertal development. However, longitudinal studies have the advantage of investigating body composition at different time points in the child's development and may give a clearer picture of the effects of the progress of the disease. There are few longitudinal studies of body composition in children with CF. Stettler et al (96) using SFT, deuterium dilution for TBW and total body electrical conductivity to measure 25, 5-10 year children with CF over 3 years concluded that statural growth, FM and FFM acquisition was slower in the CF boys compared to the control group. Using SFT the girls with CF increased in FM compared to controls. However, the control children were chosen to match the height and weight of the children with CF and therefore may not be representative of healthy children generally. Bianchi et al (65) used DXA to compare 136 patients with CF ranging in age from 3-24 years over a 2 year period. The study was predominantly investigating BMD and found deficits occurred in all of the 3 sub-groups; prepubertal, pubertal to age 18 years and adults. At baseline FM and FFM were diminished in both sexes but it is not clear from the reported data what changes occurred in body composition over time, simply that baseline and change in FFM was significantly correlated with FEV₁%. The findings of this study may not be directly comparable to other studies because the reported FFM appears to be lean mass by DXA (non-fat soft tissue) and it is not clear whether the relationship between FFM (lean mass) and FEV₁ has been size adjusted. One would expect absolute values of FFM to correlate with FEV₁ since increasing absolute FFM is related to increasing size and FEV₁ increases with increasing size.

Two longitudinal studies from Brisbane assessed BCM in 25 infants (24) and 64 children (110). The study of infants found birthweight within the normal range and although weight SDS dropped initially, after 1 year it was not significantly different from zero; length SDS was low at baseline and remained low after 1 year (mean \pm SD; -0.25 \pm 0.12). BCM SDS

was depleted initially (-1.42 ± 0.78) and within the normal range by 1 year of age; FM SDS assessed by anthropometry was low at baseline (-0.74 ± 0.38) and remained low (-1.13 ± 0.15) after 1 year. Mindful that all these infants had been identified by neonatal screening and received early treatment the findings of reduced length and low FM after 1 year are interesting. The authors suggest that increasing physical activity and pulmonary and pancreatic pathology may be a factor and that with growth, increasing BCM reaches a critical point at which energy intake becomes a limiting factor for normal rates of accretion of FM. Murphy et al (110) studied 64 children with CF aged from 5 years and found them to be well nourished assessed by BCM at baseline with only 2 children with suboptimal nutritional status and after 2 years only 5 children were assessed as suboptimal. Deterioration in BCM SDS had occurred more in boys than girls although the girls at baseline were lower than the boys (girls; 0.30 ± 0.94 , v boys; 0.76 ± 1.39). At baseline the children were also short (height SDS; mean \pm SD, -0.59 ± 0.92) and light (weight SDS; -0.32 ± 0.99) but change in height and weight SDS were not reported. There was no deterioration in FEV₁ over the study period. The authors acknowledge that the equation used to convert total body potassium to BCM may not be appropriate to either a patient or paediatric population. The finding that, in this study, boys had greater deterioration in nutritional status compared to girls is in accord with some previous studies (96;111) although it is contrary to the findings in other studies (10;112) and to expectations considering the better prognosis in males compared to females with CF (3;112). As discussed previously, it is likely that the cohort studied, who were diagnosed following neonatal screening, would have received early treatment and therefore are likely to have a better nutritional status than those children with CF who were diagnosed following symptoms of failure to thrive and persistent chest infections.

The cohort studied by Murphy et al was also part of a larger group including adolescents and adults (n=153) studied by Buntain et al (100); the children and adolescents were followed up in 2006 (36). Using DXA and measures of BCM and anthropometry the 2004 study was mainly concerned with bone status finding no bone deficits in the children, some deficit in adolescents and severe deficits in the adults with CF. They conclude that factors indicating nutritional status such as BCM and BMI, physical activity and spirometry are

positively related to BMD and days in hospital and number of admissions negatively associated with BMD. Although BCM was reported between the controls and patients the values were expressed in grams and therefore not comparable between groups since the numbers of males and females were different and the ages are not clear. The follow-up study in 2006 using DXA, only examined the children and adolescents (n=85) and found that the CF children (aged 5-10 years) were not significantly different from controls at the start but did not increase lumbar spine (LS) BMD as much as the control group. The older group (11-18 years) started with deficits of total, LS and femoral neck BMD and did not gain total body and femoral neck BMD at the same rate as the controls. In summary, the children with CF at age 5-10 years were not significantly different from the controls but over 2 years they fail to accrue bone at the same rate as the controls at the LS only. However, the older children at age 11-18 already have lower BMD at all 3 sites, fail to accrue bone at the neck of femur and total body at the same rate as controls but maintain a similar rate of gain to controls at the LS. Lean tissue mass (non-fat soft tissue) by DXA was similar at the start between children with CF and controls and accrual was similar despite a smaller weight change in the children with CF. The adolescent group had less lean tissue at the start but accrual was similar and weight change was similar. Interestingly, the adolescents with CF had a non-significant higher gain in height than the controls which does not appear to be related to timing of puberty. Pubertal stage and menarche was reported as similar in both groups of girls however, the boys with CF were reported as having delayed pubertal development at the start compared to controls and this difference persisted over 2 years. Lung function was stable in the children over 2 years but declined in the adolescents. Activity was reported as greater in the CF children at the start of the study and remained higher whereas adolescents started with a similar activity level which diminished over time compared to controls

In summary, no previous study has used a criterion technique to assess body composition in children with CF and different techniques, sexual dimorphism, whether there has been size adjustment, varying age ranges and the difference in average time of diagnosis related to neonatal screening programmes make comparison between studies difficult. Available studies suggest that deficits of FM, FFM and bone are most severe in adults and reports in

children vary between no difference from controls to depletion of either or both of FM and FFM (including bone). There is agreement that deficits become apparent with increasing age (10;95;100). BMI has been shown to be a poor indicator of nutritional status since hidden depletion of FFM has become apparent in both adults and young people. Surprisingly, since this is a disease characterised by undernutrition, obesity is increasingly becoming a problem that, with longevity is likely to be related to increasing difficulties in adult life. Sex differences have been noted, some studies reporting worse nutritional status in boys compared to girls (96;111) and some reporting that girls have poorer nutritional status than boys (10;112). There are reports of pubertal delay in girls (35) and young boys (36) and also of no pubertal delay (100).

2.5 Relationship between growth, body composition and spirometry

A seminal paper by Corey in 1988 (39) comparing growth and lung function between Boston and Toronto patients showed that although lung function was similar, growth in patients was greater and survival longer in Toronto compared to Boston patients. This was attributed to the high fat diet prescribed in Toronto. There is also evidence that improving nutrition can delay deterioration of lung function and improve survival (50;106) and that children with short stature have poorer lung function later in life (57). FEV₁ predicts mortality (113) and yearly rate of decline in FEV₁ is a better predictor of mortality (114;115). A study in the UK (58) found that FEV₁% and % IBW predicted survival at 5 years.

2.5.1 Cross-sectional reports of body composition and spirometry

Reviews of national databases of CF patients allow for the study of large numbers of patients and therefore confidence in the findings, although, typically the available data is limited to weight, height, spirometry and clinical factors. Rather than body composition, nutritional status is reported in terms of stunting or wasting. Whilst these studies are

informative the cross-sectional design cannot reveal the nature of the relationship between spirometry and nutritional status. The cross-sectional part of a study of the German CF registry data (n=3298) by Steinkamp et al (13) found that weight, height and lung function decreased with age, FEV₁ was negatively related to malnutrition and *Pseudomonas aeruginosa* infection but not related to sex and that those malnourished had lower FEV₁ and higher markers of inflammation regardless of *Pseudomonas aeruginosa* infection. Kastner-Cole et al (52) reviewed the UK registry data regarding the prevalence of overweight and obesity and emphasised the positive relationship between high BMI and lung function especially in children. However, the large age range and type of data available limits the conclusions that can be drawn from these retrospective studies. Smaller, prospective studies that distinguish between differing growth stages such as pre-pubertal, adolescent and adult and the sexes may give more information about the relationship between growth, body composition and lung function. A large study which evaluated 114 children and adolescents in 3 age groups; under 6, 6-10 and 11-18 years using DXA found the mean value for FFM SDS was < -2 in the 2 youngest groups and -4 in adolescents and was positively correlated with FEV₁ (116). Unfortunately FFM was adjusted for weight not height which would have been more appropriate given they were all short (children -0.5, adolescents, -0.9 height SDS). This may account for the very low FFM SDS noted even in the very young children and the subsequent report of a large proportion of the subjects (50-60%) with hidden depletion of FFM (% ideal weight for height >85% and FFM SDS <-2).

Many studies have low numbers, crude techniques, do not adjust for short stature or fail to address the sexes separately. Even so, a consistent positive relationship between lean mass (bone and FFM) and lung function has been noted in both adults (117-119) and children (36;65). It has been suggested that lung function is influenced by body muscle mass (8) and loss of FFM is associated with loss of diaphragm muscle (120). In addition, exercise improves lung function and sex differences in exercise habit contribute to sex differences in lung function (121). However, Ahmed et al (111) reports a positive relationship between spirometry and both FFM and FM in boys only using SFT and Pedreira et al (11) using DXA report a relationship between FM and FEV₁ (non-significant).

2.5.2 Longitudinal reports of body composition and spirometry

The mechanisms of the inter-relationship between lung function and nutritional status are complex and not completely understood and the direction of the relationship is unclear (10;11). Although it is not possible to define cause and effect, longitudinal studies have the advantage of demonstrating the relationship between changes in nutritional status and changes in clinical status.

A report of the longitudinal data from the German CF registry (13) found that in 536 children and 477 adolescents those with normal weight had a smaller decrease in FEV₁% over 2 years than those who were underweight for their height and those with improved nutritional status showed improved FEV₁%. Another large study (n=931) showed that those children at age 3 who were at or below the 5th centile for weight-for-age had poorer lung function at age 6 years compared to those who had a weight-for-age over the 75th centile at 3 years (122). Those children who had improved weight SDS had improved FEV₁% and no difference was found in growth and nutritional status between the sexes or between age 3 and 6. This is in accord with another large study (n=319) of children from 6 to 8 years (68) that found baseline weight was significantly associated with FEV₁% and those with at least a 100g weight gain per month had significant increases in FEV₁% compared to those who did not. This is further supported by intervention studies demonstrating that overnight gastrostomy feeds for just over a year stabilised spirometry in the treated group whereas in the severity matched control group FEV₁ decreased by 13% (106) and overnight feeds given for 1-2 years resulted in a reversal of declining FEV₁ (50). Zemel et al (10) performed another large analysis (n=968) using the US CF registry data for children aged 5-8 and found declining FEV₁ over 4 years with most decline in those with FEV₁ ≥ 90% at baseline. The authors suggest that young children with good lung function and intercurrent pulmonary illness may not be treated as aggressively as those with poor lung function. Deteriorating lung function was worse in girls compared to boys. This study found clear sex differences in this young age group; height SDS improved in boys but deteriorated in girls and weight SDS deteriorated in both sexes but more so in the girls.

Bianchi et al (65) investigated 136 patients with CF dividing the data into pre-pubertal, adolescent and adult subjects. Surprisingly they found that 40% had low BMD with no difference between the age groups and no difference using size adjusted BMD SDS. Change in BMD over 2 years positively predicted change in FEV₁ whereas change in FFM was only weakly related to change in FEV₁. This may, in part, be explained by the fact that the authors use 'FFM' as 'the soft tissue proportion of non-fat region'. It is likely that if bone was included in FFM the relationship with FEV₁ would be stronger.

The fact that changes in body composition are related to changes in lung function seems clear but it is difficult to draw conclusions about cause and effect. Some evidence from animal studies suggest that sub-optimal nutrition has a detrimental effect on the lung. The malnourished mouse model of respiratory infections in CF suggests that malnutrition contributes to compromised lung defences, bacterial colonisation and systemic inflammatory response (123). The timing of undernutrition has an effect in rats, in young animals causing a retardation of growth and sometimes irreversible non-development of the lung and in adults an alteration to the architecture of the terminal air spaces similar to emphysema (124). Fetal undernutrition has been suggested as a cause of lower FEV₁ in a study of men over 60; those with the lowest birthweight had the lowest FEV₁ after adjusting for current height and age (125).

It could also be argued that CF related lung disease affects body composition due to the inflammatory response to chronic bacterial infection leading to an altered protein-energy balance, lack of appetite and reduced activity. Much research into the relationship between severity of lung disease, protein catabolism and systemic inflammation and the detrimental effect on FFM including bone has been undertaken by a group of researchers from the University of Wales College of Medicine. Using measures of systemic inflammation (serum tumour necrosis factor- α and interleukin-6), protein breakdown (urinary pseudourine) and connective tissue breakdown (cross-linked N telopeptides of type 1 collagen) they found that inflammatory mediators were higher in clinically stable adults with CF compared to controls and were related to increased protein and connective tissue breakdown which were in turn related to impaired FEV₁ and low FFM as measured by

DXA (118). This finding of inflammatory and catabolic response is supported by other studies of patients with CF concluding that chronic lung disease is a major factor in altered body composition and metabolic complications (16;126;127). Similarly for patients with chronic obstructive pulmonary disease (chronic bronchitis and emphysema) lung damage is followed by an inflammatory response and narrowing of the airway and typically weight loss (128). Studies of patients with CF suggest that loss of FFM generally will impact on inspiratory lung muscle and its function adding to the difficulties of breathing (120;129-131) and may reduce response to antibiotic treatment (132) and therefore generate a vicious cycle of pulmonary inflammation and progressive lung disease (133).

Insulin-like growth factor (IGF-1) is an important hormone that has an anabolic effect on the body by promoting protein synthesis and inhibiting protein degradation (134). Increase in muscle mass has been demonstrated where IGF-1 has been given to patients (135) and animal studies have shown that IGF-1 prevents diaphragm atrophy due to malnutrition (136). A study by Sermet-Gaudelus et al. (105) of 24 children with CF found that those with a reduction of ≥ 1 SDS lean mass by DXA after 1 year had the lowest levels of IGF-1 at baseline and that this was independent from weight and FM. They conclude that reduction of IGF-1 may not only reflect but contribute to overall nutritional status. Growth hormone stimulates linear growth and is a potent anabolic agent and has been used clinically to improve nutritional status and protein catabolism in acquired immune deficiency syndrome and burns patients (137;138). Its use in patients with CF has been shown to improve growth and clinical status (139) and protein catabolism (140) in a randomised clinical trial in pre-pubertal children.

It is likely that the relationship between lung function and body composition is not simply one of cause and effect but more about inter-dependence, deterioration in either impacting on the other via complex body mechanisms.

2.6 Summary

- Age at diagnosis impacts on clinical outcome.
- Historically, growth (weight, height and BMI) has been related to morbidity and mortality, however, the impact of body composition cannot be determined using these measurements. BMI cannot detect hidden depletion of lean mass and may not correctly identify obesity.
- There is deterioration in growth, nutritional status and body composition in patients with CF over time which may start at a very young age. Although research has shown that CF has a detrimental effect on growth, studies have variously found either no, or deficits in FM, FFM and bone in children and young people with CF.
- Sex differences are apparent but there is no agreement between studies. Given the poorer prognosis in females this merits attention.
- There is not agreement about whether pubertal delay occurs and in which sex.
- Consistent findings of a relationship between BMI or FFM (including BMD) and spirometry are reported.
- The differing methodologies in past studies make comparison difficult. Cross-sectional observational studies have limited utility in defining the multi-factorial relationship between spirometry and body composition. Longitudinal studies have the advantage of the ability to relate change in body composition to change in spirometry.

Table 2.1. Studies of body composition and lung function in patients with cystic fibrosis.

Only details relating to body composition and lung function are noted in the table.

Study	N Age yrs	Duration/ details	Outcome variables	Adjusted	Sexes separate	Findings
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Cross-sectional studies

Steinkamp 2002 (13)	3298 ≥ 2	Cross-sectional German CF registry	Weight, height, FEV ₁ , PA		Yes	Weight, height and lung function decrease with age. FEV ₁ related to malnutrition & PA but not to sex at all ages Patient with malnutrition had ↓FEV ₁ , ↑inflammation independent of PA
Kastner- Cole 2005 (52)	2987 1-56	UK CF registry Homo ΔF 508	Weight, height, BMI, FEV ₁		Yes	Prevalence overweight; children, 8% M, 10% F, adults 13% M, 5% F Prevalence obesity; children, 1% M&F, adults, 1.6% M, 0.2% F Children; BMI SDS +ve association with FEV ₁ % through entire range of BMI (-4 to +3) Adults; + ve association with FEV ₁ until BMI 23 kg/m ²
Rochat 1994 (119)	12 19-24	Cross-sectional malnourished CF vs controls	DXA LM, BMC, FM, FEV ₁ Weight	Height normalised to 170cm	No	↓BMC & LM FEV ₁ correlates with weight, LM & BMC but not FM
Stallings 2005 (72)	16 females 8-29	Cross-sectional Pre & post puberty, CF vs controls	DXA FM, FFM arm anthropometry FEV ₁ REE	None	Only females	CF ↓height, arm muscle area, ↑REE but not different in FM & FFM BMI +ve association with FEV ₁ and age – ve association with FEV ₁ Conclude; poor growth, nutritional status and delayed menarche associated with poorer lung function
King 2010 (117)	86 19-59	Cross-sectional CF vs ref data	DXA FFM, BMI, FEV ₁	Height	Yes	FFM depletion in 14%, low BMI in 18.6% FEV ₁ associated with FFM in both sexes
Pedriera 2005 (11)	50 7-17	Cross-sectional Mild lung disease CF vs ref data	Weight, height, BMI DXA FM, FFM	None	No	BMI SDS strong +ve association with FEV ₁ FFM SDS weaker significant association with FEV ₁ FM SDS non-significant +ve association with FEV ₁

FEV₁; forced expiratory volume in 1 sec, PA; pseudomonas aeruginosa, BMI; body mass index, M; male, F; female, SDS; standard deviation score (Z), DXA; dual-energy X-ray absorptiometry, LM; lean mass (non-bone), CF; cystic fibrosis, BMC; bone mineral content, FM; fat mass, FFM; fat-free mass, REE; resting energy expenditure, BMD; bone mineral density, BCM; body cell mass, LS lumbar spine, %IBW; percent ideal body weight, BIA; bio-electrical impedance analysis, SFT; skinfold thickness, TBW; total body water, FVC; forced vital capacity, TOBEC; total body electrical conductivity.

Table 2.1 continued. Studies of body composition and lung function in patients with cystic fibrosis.

Only details relating to body composition and lung function are noted in the table.

Study	N Age yrs	Duration/ details	Outcome variables	Adjusted	Sexes separate	Findings
Cross-sectional studies continued						
Henderson 1999 (64)	40 5-20	Cross-sectional Pair-match	DXA BMD, FM, LM	None	No	Mean weight and height SDS -0.7. Reduction of 19% BMC, 18% FM and 12% LM. Weak correlation LM and FEV ₁ Large age range
Buntain 2004 (100)	153 5-56	Cross-sectional Child Adolescent Adult CF vs controls	BMD, BCM, FEV ₁	Height Sex	No	Adolescents ↓ weight & height, adults ↓ weight LS & total BMD ↓ adolescents and adults BCM ↓ in child/adolescents Children & adolescent; BMI, BCM and FEV ₁ predicted BMD Adults: BCM & no. of days in hospital predicted BMD No pubertal delay
Sermut-Gaudelus 2007 (116)	114 2-18	Cross-sectional CF vs ref data <6, 6-10 11-18yrs	DXA BMD, FM,FFM	Weight	No	All 3 groups had FFM SDS < -2 (adolescents -4), FM was > 0 in the 2 youngest groups and -1.1 in adolescents. All were short <-0.5 with adolescents shortest -0.9 FFM SDS significantly correlated with FEV ₁ . LS BMD <-1 in all groups. Mean FEV ₁ declined after age 10, osteocalcin correlated with FEV ₁ and FFM 50-60% normal weight (%IWH > 85%) had FFM SDS < -2 ie hidden depletion FFM Pubertal delay in both sexes.
Ionescu 2002 (118)	40 23±0.8	Cross-sectional CF vs control	DXA FM, FFM FEV ₁	Height	No	Clinically stable adults catabolic and this related to lung disease severity, systemic inflammation and body composition FEV ₁ related to FFM Those with low FFM had lowest FEV ₁
Ionescu 2003 (104)	56 17-38	Cross-sectional CF vs control	DXA FM, FFM BIA FFM Lung function	Height	No	Apparent or hidden loss of FFM related to overall disease severity. Hidden depletion of FFM associated with ↑ loss BMD and systemic inflammation FEV ₁ related to BMI and FFM

FEV₁; forced expiratory volume in 1 sec, PA; pseudomonas aeruginosa, BMI; body mass index, M; male, F; female, SDS; standard deviation score (Z), DXA; dual-energy X-ray absorptiometry, LM; lean mass (non-bone), CF; cystic fibrosis, BMC; bone mineral content, FM; fat mass, FFM; fat-free mass, REE; resting energy expenditure, BMD; bone mineral density, BCM; body cell mass, LS lumbar spine, %IBW; percent ideal body weight, BIA; bio-electrical impedance analysis, SFT; skinfold thickness, TBW; total body water, FVC; forced vital capacity, TOBEC; total body electrical conductivity.

Table 2.1 continued. Studies of body composition and lung function in patients with cystic fibrosis.

Only details relating to body composition and lung function are noted in the table.

Study	N Age yrs	Duration/ details	Outcome variables	Adjusted	Sexes separate	Findings
Longitudinal studies						
Shepherd 1986 (50)	10 3-13	1 yr supplementary feeding	Weight, growth Infections FEV ₁ FVC		No	Weight↑, height ↑ and fewer infections Reversal of lung function decline Initial catabolic state → anabolic by 6-12 months
Zemel 2000 (10)	968 5-8	4 yr longitudinal US CF registry	Weight, height, %height for ideal weight, FEV ₁		Yes	Decline in FEV ₁ % over time with most decline in those highest at start ≥90% and girls > boys. Baseline weight SDS, height SDS +ve association with FEV ₁ % Sex difference; Δ height SDS boys ↑, girls ↓, Δ weight SDS ↓ for both but girls > boys
Steinkamp 2002 (13)	536 6-12 477 12-18	Longitudinal 1 or 2 German CF registry	Δ weight/height Δ FEV ₁		No	1 yr Δ weight/height: ↓→↓FEV ₁ , normal & stable → stable FEV ₁ , ↑→↑FEV ₁ 2 yr child & adolescents with normal nutrition had smaller decreases in FEV ₁ than malnourished Fall in weight/height ≥5% predicted associated with ↓FEV ₁ and vice versa Improved weight/height → = or ↑ FEV ₁
Konstan 2003 (122)	931 3yrs	3yr longitudinal US & Canada	Weight, height Lung function		Yes	Weight and height SDS -0.5 at age 3 & 6 yrs No difference in growth & nutritional status between sexes and at 3 & 6 yrs Weight, height status at age 3 strongly associated with lung function at 6 yrs. Δ weight ↑ → ↑ lung function
Peterson 2003 (141)	319 6-8	2 yr longitudinal	Weight, FEV ₁		No	1kg higher initial weight→ 55ml ↑ FEV ₁ 2yr 1 kg weight gain → 32ml ↑ FEV ₁
Ahmed 2004 (111)	143 2-18	1-8 yr longitudinal CF vs controls	Weight, height, BMI, SFT, FEV ₁	Weight	Yes	Weight, height & BMI SDS ↓ decreasing with age Girls < FM till 13yrs and then the same, <FFM at all ages Boys <FM and FFM especially in late puberty. Boys FEV ₁ +ve association with FM & FFM & -ve association with age Girls no association of body composition and FEV ₁

FEV₁; forced expiratory volume in 1 sec, PA; pseudomonas aeruginosa, BMI; body mass index, M; male, F; female, SDS; standard deviation score (Z), DXA; dual-energy X-ray absorptiometry, LM; lean mass (non-bone), CF; cystic fibrosis, BMC; bone mineral content, FM; fat mass, FFM; fat-free mass, REE; resting energy expenditure, BMD; bone mineral density, BCM; body cell mass, LS lumbar spine, %IBW; percent ideal body weight, BIA; bio-electrical impedance analysis, SFT; skinfold thickness, TBW; total body water, FVC; forced vital capacity, TOBEC; total body electrical conductivity.

Table 2.1 continued. Studies of body composition and lung function in patients with cystic fibrosis.

Only details relating to body composition and lung function are noted in the table.

Study	N Age yrs	Duration/ details	Outcome variables	Adjusted	Sexes separate	Findings
Longitudinal studies continued						
Zemel 1996 (67)	26 5-10	3 yr longitudinal CF vs control mild lung disease	Weight, height, arm anthropometry, TBW(FFM), FEV ₁ , FVC, REE	FM weight FFM none	Yes	↑REE not associated with declining lung function Sex differences; girls REE increased, boys lung function decreased in 3 yr, Δ height SDS ↓ in girls and boys. %IBW and genotype predict Δ lung function REE adjusted for FFM was CF>controls, CF boys>CF girls, the differences increasing with age. No account of the relationship between body composition and lung function
Stettler 2000 (96)	25 5-10	3 yr longitudinal CF vs control matched for weight mild lung disease	SFT, TBW(FFM), TOBEC	None	Yes	Sex difference; boys Δ height SDS ↓, ↓FFM, ↓FM and girls Δ height SDS ↓, ↑FM compared to controls who are atypical. No account of the relationship between body composition and lung function
Buntain 2006 (36)	85 5-18	2 yr longitudinal 5-10 11-18 CF vs controls	BMD, BMC, DXA LM FEV ₁ , FVC	Height Sex	No	At start weight and height is similar in children and ↓ for CF adolescents Δ weight ↓ children and same in adolescents who had NS ↑ Δ height At start no BMD deficit in children but Δ LS BMD ↓ compared to controls At start adolescents BMD ↓ compared to controls but rate of change the same for LS and ↓ for total and femoral neck BMD Children; FVC related to total BMD & LM related to LS BMD Adolescents; FEV ₁ & LM related to total and LS BMD
Bianchi 2006 (65)	136 3-24	2 yr longitudinal Pre-pubertal child Adolescent Adult	BMC, BMD DXA FM, LM	For 'body size' Sex		40% had BMD SDS < -2 no significant difference between age groups even when size adjusted BMD correlated with FEV ₁ and Δ BMD correlated with Δ FEV ₁ At start both FM & LM ↓, LM correlated with FEV ₁ Δ FFM weakly correlated with Δ FEV ₁ No sex differences

FEV₁; forced expiratory volume in 1 sec, PA; pseudomonas aeruginosa, BMI; body mass index, M; male, F; female, SDS; standard deviation score (Z), DXA; dual-energy X-ray absorptiometry, LM; lean mass (non-bone), CF; cystic fibrosis, BMC; bone mineral content, FM; fat mass, FFM; fat-free mass, REE; resting energy expenditure, BMD; bone mineral density, BCM; body cell mass, LS lumbar spine, %IBW; percent ideal body weight, BIA; bio-electrical impedance analysis, SFT; skinfold thickness, TBW; total body water, FVC; forced vital capacity, TOBEC; total body electrical conductivity.

Chapter 3. Body composition measurement techniques in children and adolescents

3.1 Introduction

The gold standard for body composition measurement at the molecular level is cadaver analysis, whereas in vivo techniques do not measure body composition directly but rather predict it from measurement of other body properties (142). Consequently methodological error whilst acquiring the raw data and theoretical error when converting raw data to body composition variables must be considered when assessing the value of techniques.

There is increasing interest in measuring body composition because simple measures of weight and height cannot distinguish between predominantly metabolically active tissue (FFM) and predominantly storage tissue (FM). The issue is most pertinent in patients in whom the magnitude of specific components of body composition impact on disease progression and management. For example, calculating drug dose based on weight in a normal weight child with low FFM and therefore high FM could mean that the dose is not metabolised as fast as expected (143). The problem of accurate assessment of body composition in children is made more difficult because with growth and chemical maturation of the lean tissue, the relative proportions of protein, mineral and water change at different rates in individuals and between the sexes. In addition, for patients there may be a disruption to the 'normal' proportions of the constituents of lean mass.

Investigation of body composition in children with CF may be beneficial because of the potential to; (i) aid understanding of the mechanisms that underlie deficits in patients with CF, (ii) assess the effectiveness of medical and nutritional interventions and (iii) identify those children at most risk of deterioration.

Wang (144) proposed a structural framework for studying body composition describing 5 levels of increasing complexity; I) atomic, II) molecular, III) cellular, IV) tissue system and V) whole body (see **Figure 3.1**). Information can be transformed between levels, for example, using the chemical constant required to convert nitrogen to protein (Level I to II) or the density of the whole body to derive FM (Level V to II). This chapter reviews the merits of the most commonly used methods available to assess growth and body composition in children and adolescents.

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Figure 3.1. Five levels of body composition (Wang 1992).

3.2 Simple measures

3.2.1 Weight, height and body mass index (Level V)

Although weight, height and BMI are commonly used to classify growth by use of centile charts or by SDS the measures do not actually distinguish the different components of body weight. There is generally an assumption that BMI relates to fatness without consideration to the amount of lean tissue present. Therefore in normal or low BMI patients with low FFM, for example, some patients with CF, the BMI categorisation masks the high FM component which may be putting them at higher risk of metabolic syndrome. Indeed Wells et al.(145) reports data on patients with congenital myasthenia where a low BMI masks high FM (by isotope dilution and SFT) **Figure 3.2**

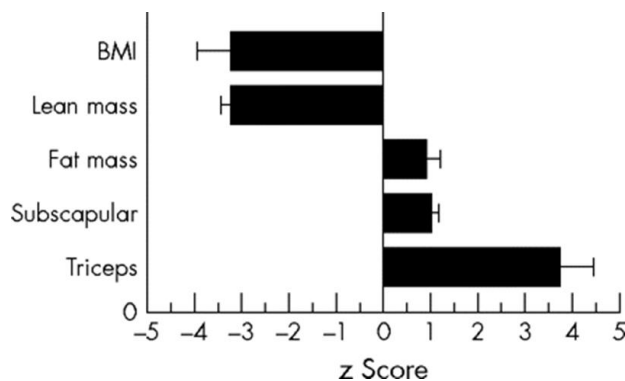


Figure 3.2. “Data from infant patients with congenital myasthenia, a condition in which the development of connective tissue is impaired. Despite extremely low BMI SDS, the patients have body fat levels higher than the average in healthy children. This paradox can be attributed to extremely low levels of lean mass. Though they are underweight, energy intake is not itself constraining their growth”. Wells et al.(146)

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Another study by Wells et al. (147) (**Figure 3.3**) shows two-fold variation in fat for a given BMI in individual children.

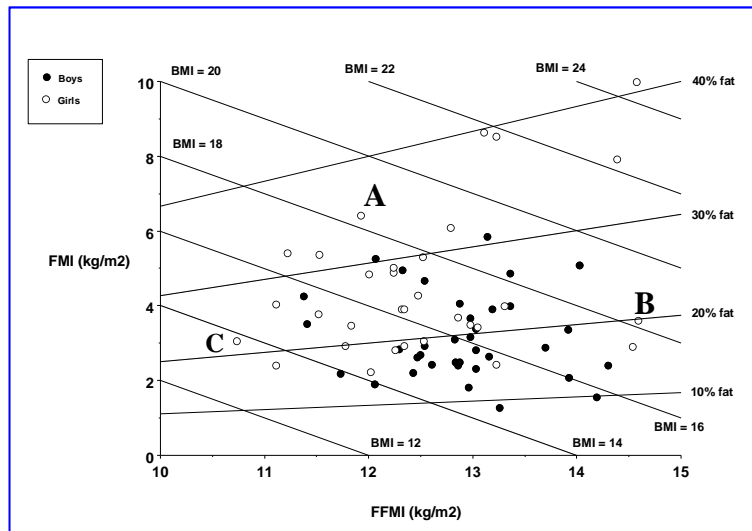


Figure 3.3. “Hattori graph for children aged 8 showing fat mass adjusted for height (fat mass index; FMI) v fat-free mass adjusted for height (fat-free mass index; FFMI). The individuals A and B represent 2 girls with similar BMI (approximately 18 kg/m²) but with A having twice the FMI of B. The individuals B and C have similar percent fat, but very different BMI and FFMI” (Wells et al (148).

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Similarly, BMI is not a reliable predictor of lean tissue either (149) and there are several reports of hidden depletion of lean mass (low lean with normal BMI) in adults (104;117) and children (105;150). Although height and weight indices are commonly used to calculate weight for height and height for age in paediatric patients, rarely is genetic potential or pubertal delay, which is common in children with CF, taken into account.

3.2.2 Regional skinfold thickness (Level IV)

SFT measurement is a cheap, quick and simple technique to rank relative fatness and assess specific subcutaneous fat depots. The measurements are made usually at 3 or 4 sites by ‘pinching’ a skinfold, pulling it away from the underlying muscle and then measuring using a calliper.



Figure 3.4. *Measurement of bicep skinfold thickness*

There is an assumption that subcutaneous fat is proportional to total body fat although it does in fact vary with age, sex and ethnicity (151). Inter and intra-operator error in general is low compared to individual variation but it is a technique that requires experience. In obese individuals the precision and accuracy is poorer. This technique is inappropriate to derive FFM (weight-FM) because only properties of FM are assessed.

The measurements are usually made at several sites most commonly of the overlying skin of the sub-scapular, supra-iliac, bicep and tricep sites. Other sites may be used but may be more difficult to measure. The raw measurements can then be used to rank fatness or converted to SDS (152;153) to allow longitudinal assessment of regional fatness. Until recently the SFT reference data was old (154) but more contemporary data (155) has been used in this study although not yet generally available.

3.2.3 Waist circumference

Waist circumference is a quick and easy measurement that has been shown to relate to visceral adiposity (156;157). In adults, increased waist circumference has been

shown to relate to obesity related diseases such as cardio-vascular disease (158) and even in children a relationship with adverse blood lipoprotein profile (159;160). Waist circumference is a better predictor of central adiposity than waist to hip ratio (142) and waist to height ratio is more closely linked to morbidity in childhood than BMI (161). There is a dearth of studies investigating the relationship between FFM and waist circumference but a large study of adults concluded that increased waist circumference was more closely associated with increased FM than FFM in both men and women (162).

3.3 Predictive techniques

Prediction techniques are those which depend on measuring some characteristic of the body to predict components of body composition.

3.3.1 Skinfold thickness to derive whole body fat

SFT measurements may be used in the raw state or converted to SDS as described above or may be used as a predictive technique by applying appropriate equations (151;163-165). The use of predictive equations is problematic because the equations; (i) are population specific and have generally been derived in healthy white populations, (ii) convert individuals to the average and (iii) are derived by regression using some other body composition method which typically, has not been a criterion technique. Consequently accuracy in individuals is poor (limits of agreement $\pm 9\%$ fat) and varies with magnitude of fatness (166;167).

3.3.2 Bioelectrical impedance analysis (Level V)

Single frequency BIA is a quick, easy, non-invasive technique where a small alternating current is passed through the body from one electrode to another (at hand and foot) and the voltage drop (by which impedance is derived) is measured by 2 more electrodes. It is based on the principle that FFM contains water and

electrolytes and therefore acts as a conductor whereas FM is relatively anhydrous and therefore resists the flow of the current.



Figure 3.5. Bioelectrical impedance analysis

Impedance (Z) is the frequency dependent opposition of a conductor to the flow of an alternating electrical current and is composed of resistance (R) and reactance (X_c). Biological conductors have both resistive and reactive components, however, the reactive component is so small ($<4\%$ of Z) that R is assumed to be equivalent to Z . The theoretical model also assumes that the body is an isotropic cylinder of constant width, with its length proportional to the subject's height.

The resistance of the cylinder is represented by;

$$\mathbf{Z = K * HT/A} \qquad \qquad \qquad \mathbf{Equation\ 3.1}$$

Where Z is impedance, K is a constant, HT is the length of the cylinder and A is the cross-sectional area of the cylinder.

Alternatively;

$$\mathbf{A = K* HT/Z} \qquad \qquad \qquad \mathbf{Equation\ 3.2}$$

Since;

$$\mathbf{HT * A = Volume} \qquad \qquad \qquad \mathbf{Equation\ 3.3}$$

Multiplying both sides of the equation by HT;

$$\mathbf{Volume = K * HT^2/Z} \qquad \qquad \qquad \mathbf{Equation\ 3.4}$$

The volume or TBW can therefore be calculated from HT and Z once K has been calculated for that specific population. K is calculated by regressing Z on TBW measured by a criterion technique.

However, the body is not an isotrophic cylinder and regional body weight does not match distribution of impedance (168;169) since long thin cylinders such as the arms have more resistance than the wider cylinder of the trunk. This is a problem where proportions differ in growing children and patients with altered proportions (142). One solution is to divide the body into segments of similar sized cylinders (trunk, right and left arm and leg) and measure the segments separately, thereby improving accuracy. In addition, the slope of the regression equations between height and Z may be affected by sex, age and other characteristics and therefore need to be calculated for specific populations. This is particularly pertinent in patient groups and although whole body equations have been derived for some patient groups (89;92;170;171) the accuracy in individuals is poor and may be affected by clinical status. In healthy populations error is typically $\pm 8\%$ fat (167) and Puiman (92) reports an underestimation of 6% of TBW in patients aged 4 to 18 yrs with CF.

Since K is proportional to fluid volume and inversely proportional to the number of free ions, changes in geometry (volume), electrolyte concentration or body temperature can affect Z (172).

The use of prediction equations, either those used for the equipment print-out or those derived in specific populations require an assumption that each individual has an impedance value equal to the mean and thereby may introduce error. An alternative to this method is to use the raw impedance data (height^2/Z) and convert this to SDS compared to a reference population (155).

3.3.3 Total body conductivity (Level V)

Total body electrical conductivity is similar to BIA in that the theoretical assumption is that body electrical conductivity predicts body composition (173). The body is passed through a coiled wire (solenoid) and changes in the electromagnetic field are measured to indicate the conductivity of the tissues and thereby TBW and FFM.

However, the instrument is bulky and affected by environmental factors and hydration of the subject and performs best after a 6 hr fast, making it unsuitable for paediatric use. Manufacture of devices stopped in 1994 and therefore availability is limited.

3.4 Two-component models (2CM)

The two-component (2CM) divides body weight into FM and FFM the former being homogenous and the latter although heterogeneous, for the purpose of 2CM is assumed to be of constant density. Accuracy depends on the assumption of constancy of the FFM which depends on sex, age, pubertal development, ethnicity and disease state. Therefore, wherever possible population specific values for the nature of the FFM should be used.

3.4.1 Hydrometry (Level II)

Total body water can be measured on the principle that it is equal to the amount of stable isotope added to the body divided by the concentration of the isotope in the water compartment of the body after the equilibrium period (174). Deuterium oxide ($^2\text{H}_2^{16}\text{O}$) is commonly used but other stable isotopes can also be used such as ^{18}O -oxygen. The fact that deuterium oxide differs from ordinary water ($^1\text{H}_2^{16}\text{O}$) by 1 neutron means that it is quantifiable by mass spectrometry. Once ingested the hydrogen atoms of deuterium are exchangeable with the hydrogen atoms of the water pool (175). The equilibrium period depends on the sample of body fluid used; blood sampling is problematic in children, urine sampling is unsatisfactory because it is difficult to predict a time when the urine content of the bladder is typical of the whole body water and therefore saliva samples are the best option for all except babies. The equilibrium period is longer for urine (4-6hrs) than saliva (3-4 hrs) (176), although the saliva equilibrium period is extended in the obese (177;178).

The calculation of TBW will be affected by any isotope leaving the body during the early equilibrium period due to sweating or voiding although such loss is minimal and not considered in calculation of TBW. Drinks taken do need to be accounted for as they will dilute the isotope leading to a lower concentration and therefore overestimation of TBW.

The equation is:

$$N = \frac{TA}{A} * \frac{(Ea-Et)}{(Es-Ep)} \quad \text{Equation 3.5 (179)}$$

Where N = dilution space (ml)

T = amount of tap water in which portion a is diluted (ml)

A = amount of isotope (g)

a = portion of dose used in analysis (g)

E = isotopic enrichments in delta units relative to an international standard,
Vienna Standard Mean Ocean Water (VSMOW)

Ea enrichment of portion of dose

Et enrichment of tap water used

Es enrichment of post dose sample

Ep enrichment of pre-dose sample

There is a difference between TBW and dilution space measured by isotopic exchange due to non-aqueous exchange of either 18-oxygen or deuterium with oxygen and hydrogen ions (180;181). This is addressed by applying a correction factor;

$$\begin{aligned} \text{TBW} &= N/1.044 \text{ in mls for deuterium} \\ &= N/1.01 \text{ in mls for 18-oxygen (180)} \end{aligned}$$

Once TBW has been calculated the amount of fluid consumed is subtracted to avoid an overestimation of water and consequently overestimation of FFM. FFM is calculated using age and sex specific constants for hydration of FFM;

$$\text{FFM} = \frac{\text{TBW}}{\text{Hydration of FFM}} \quad \text{Equation 3.6}$$

The constant for adults is considered to be 0.73 and for babies around 0.80 but the relationship for the period between the two is not linear (182). Fomon's landmark paper in 1982 and Lohman's later merging of data with that of Fomon (183) has been

widely used in calculating FFM however, not all age points were measured, extrapolation being used for the missing time points. Recently, I collected data for a comprehensive study of more than 500, 4 to 22 year old subjects that provided measured hydration factors throughout the range (184). The same study provided the data to generate SDS for TBW. Using a SDS for an individual avoids the problem of introducing an error by assuming they have an average value for hydration when converting TBW to FFM.

3.4.2 Densitometry (Level V)

Densitometric techniques are based on the principle that, if the density of two components are known and the overall density of the two components combined is known then it is possible to work out the proportion of one to the other. Body volume and mass are measured to calculate overall density and then assumed densities of FM and FFM used to calculate %fat:

$$\% \text{ fat} = (C1/\text{body density} - C2) \times 100 \quad \text{Equation 3.7}$$

$$\text{Where } C1 = \frac{(\text{density}_{\text{lean}} \times \text{density}_{\text{fat}})}{(\text{density}_{\text{lean}} - \text{density}_{\text{fat}})}$$

$$\text{And } C2 = \frac{\text{density}_{\text{fat}}}{(\text{density}_{\text{lean}} - \text{density}_{\text{fat}})}$$

For adults, the density of fat is considered to be 0.9007 kg/L and the density of lean 1.100 kg/L

Therefore:

$$\% \text{ fat} = (495/\text{body density}) - 450 \quad \text{Equation 3.8 (185)}$$

Fat is homogenous and therefore assumed to be of constant density (186) whereas lean tissue is heterogeneous, the relative amounts of protein, mineral and water varying with growth and different between the sexes. This is a severe limitation when using densitometric techniques to measure children. Until recently reference

data for children has been sparse and based on small numbers of limited age range (182;187) although our recent reference data for 4 to 22 years from our centre should improve the accuracy of prediction of FM and FFM (155;184).

3.4.2.1 Under-water weighing/ hydro-densitometry

This technique requires the person to be weighed and then completely immersed in water whilst being weighed again.

Image removed for copyright purposes

Figure 3.6. Under-water weighing apparatus.

Based on Archimedes principle whole body density can be calculated:

$$\frac{\text{Density of an object}}{\text{Density of the fluid}} = \frac{\text{Weight}}{\text{Weight} - \text{apparent immersed weight}}$$

The total immersion in a tank of water means that this technique is not suitable for the young, sick or infirm and is not suitable for field studies.

3.4.2.2 Air-displacement plethysmography (ADP)

Figure 3.7. Air-displacement plethysmography
(Boodpod; Cosmed, Rome, Italy)

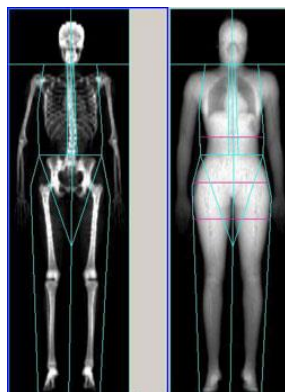


A newer technique to assess body density relies on Boyle's Law of the relation between the volume, pressure and temperature of gases. The volume of gas in the front measurement chamber is measured; (i) empty, (ii) with the 50 L calibration cylinder and (iii) with the subject inside. The front chamber is connected to the rear reference chamber by oscillating diaphragms which induce small pressure changes. Raw body volume is calculated from the volume of air without and with the subject present and then an adjustment made for residual lung volume and surface area artefact generated by the warmer air in contact with the skin. The residual lung volume may be measured but the technique is very difficult for children to perform and therefore predicted volume is often calculated. The equations used to calculate lung volume, surface area artefact and % fat in the machine's software are not appropriate for children. When measuring children the raw body volume is adjusted using age and sex appropriate predicted residual lung volume (188;189) and surface area artefact (190).

ADP has been shown to correlate well with under water weighing (191;192), however, ADP has better precision (191;193;194). The equipment is large and requires a stable temperature in the measuring room making it unsuitable for field studies. The technique requires the subject to sit still inside the machine for several periods of about a minute and is therefore not suitable for very young children (<4yrs) or those who are claustrophobic. In the last few years new equipment has

allowed for the measurement of whole body density in infants (<8kg) which, although similar in principle, is able to accommodate movement and crying (PEA POD; Cosmed, Rome, Italy).

3.4.3 Dual-energy X-ray absorptiometry (DXA; Level V)



Figure



3.8.

Dual-energy X-ray absorptiometry (Lunar Prodigy, GE Medical Systems, USA)

DXA, first developed for assessment of bone mass has been shown to be both precise and accurate when used for this purpose. Over the past decade, DXA has increasingly been used to assess body composition in research and clinical practice due to the additional information on total FM and FFM and their distribution in the trunk and upper and lower limbs. Its rapid uptake may be attributed to its ease of use, availability, and low radiation exposure (less than $2\mu\text{Sv}$ per scan), which is lower than background radiation. However, although precision and accuracy for bone is good ($<1\%$) and precision of soft tissue assessment is good ($<2\%$) (195) accuracy is variable (102;196;197). The principle on which DXA is based is that different tissues attenuate the photons passing through the body differently. Bone has high attenuation, lean tissue medium and fat low attenuation. Pixel-by-pixel estimation of body composition using a series of assumptions is complicated by the fact that wherever there is bone in the pixel the software is unable to determine the nature of the soft tissue. More than 40% of a whole body scan contains bone (196) and therefore in those pixels the machine cannot distinguish between FM and non-

bone FFM and an assumption is made that this tissue is in similar proportions to the half of the body that does not contain bone in the pixels. This is one possible source of error and studies find that assessment of soft tissue is subject to bias according to sex, size, fatness, and disease state of those being measured, which indicates that DXA is unreliable for patient case-control studies and longitudinal studies of persons who undergo significant changes in nutritional status between measurements (102;198). The large size and cost of DXA limit its use in non-hospital settings.

3.5 Multi-component models (Level II)

2CM techniques overcome the limitations of the predictive techniques by addressing both components of body weight, FM and FFM. FM is homogenous but FFM, composing of mainly protein mass (PM), MM and water varies in the relative proportions of each depending on growth and maturation and is different in the sexes and may be disrupted in disease. To improve accuracy of quantifying both components it is possible to measure one or two of the components of FFM.

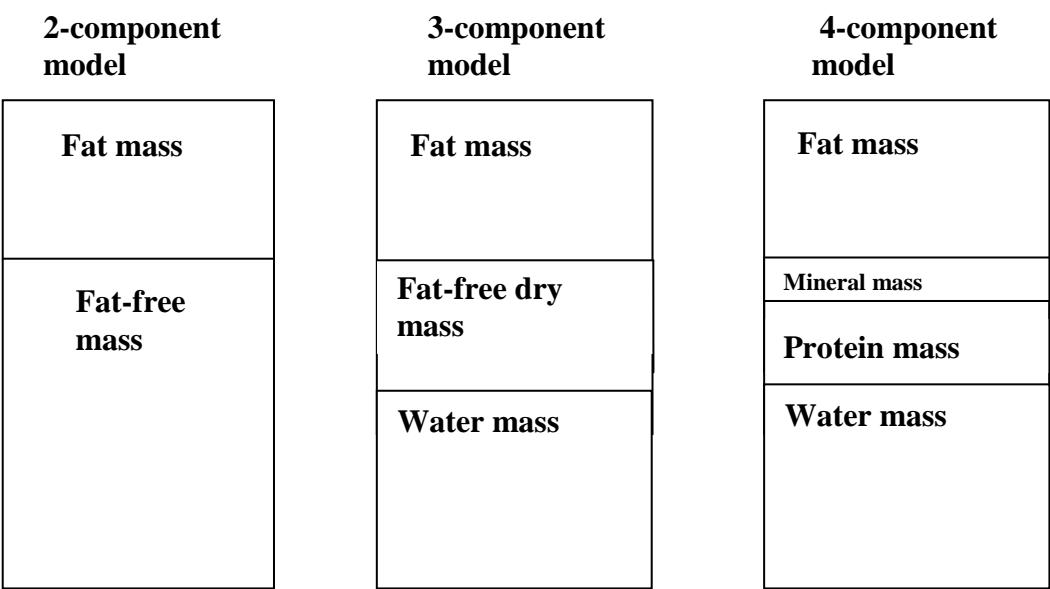


Figure 3.9. Multi-component models of body composition

The 3-component model (3CM) utilises the measurement of total body water and thereby removes the assumption about constant values for the hydration of FFM although assumes a constant ratio of PM to MM (199). The 4CM used in this study additionally utilises the measurement of mineral by DXA and thereby overcomes the assumptions of the 3CM, although there is still an assumption about the ratio of osseous to non-osseous mineral (199). The measurement of water and mineral and overall density quantify both FM and the components of FFM more accurately and allow for calculation of the hydration of FFM and ratio of PM to MM ratio.

The 4CM is considered a ‘criterion’ method for in vivo techniques because it is the most robust to detect inter-individual variability in FFM as well as accuracy over a range of FM (200).

$$\text{FM (kg)} = 2.747 \text{ BV} - 0.710 \text{ TBW} + 460 \text{ BMC} - 2.050 \text{ BW}$$

Equation 3.9 (167;199)

BV = body volume (L) from ADP adjusted for surface area artefact and lung volume

TBW = total body water (L) from deuterium dilution

BMC = bone mineral content (Kg) from whole body DXA

BW = body weight (Kg)

3.5.1 Propagation of error for measurements of FM and FFM

One might presume that using measurements from several techniques would introduce additional error. This was examined by Wells and Fuller (167;194) (see **Table 3.1**) and it is clear that the sum of errors associated with multiple measurements does not mean overall precision is poor. On the contrary, multi-component models are more accurate because assumptions about the nature of the FFM are minimal.

Error in measurement may be due to methodological variation, biological variation and a combination of both. Propagated error is the total observed error as a function

of the independent and additive technical errors (methodological and biological) and is represented by the formula;

$$V_t^2 = V_m^2 + V_b^2 \quad \text{Equation 3.10}$$

Where V_b is the biological variation, V_m is the methodological variation and V_t is the total observed variation.

Table 3.1. Propagation of error on fat mass and fat-free mass values from different models of body composition.

	Error calculating FM/FFM (Kg)	% FM	%FFM
4C model	0.22	3.1	0.50
3C model	0.20	2.8	0.50
2C models:			
Deuterium	0.27	3.8	1.3
UWW	1.00	13.8	3.8
BOD POD	0.30	5.2	1.1
DXA	0.20-0.40	3.0	1.5

From Wells et al (167) and Wells and Fuller (194). "The error is calculated from 30 children aged 5-16 years; average weight assumed to be 48.7 kg (41.5 kg FM and 7.2 kg FFM). FM; fat mass; FFM; fat-free mass; UWW; under-water weighing; DXA; dual-energy X-ray absorptiometry. 4CM uses deuterium, BOD POD and DXA; 3CM use deuterium and BOD POD".

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3.6 Other techniques

3.6.1 Magnetic resonance imaging (Level V)

Image removed for copyright purposes

Figure 3.10. Magnetic resonance imaging equipment

Magnetic resonance imaging (MRI) is a technique which measures volume rather than mass. Images are produced as a result of the alignment of hydrogen protons in water or fat either with, or against a strong magnetic field around the body. The absorption or emission of energy by the protons are then analysed to produce cross-sectional images of the body which can be used to calculate tissue volumes.

Despite good quality imaging, comparison with other techniques is difficult because MRI quantifies adipose tissue not FM. The proportion of fat in adipose tissue is variable and it is therefore difficult to calculate FM. The advantage of MRI is that it can identify regional body composition and is the only current viable technique for identifying intra-abdominal adipose tissue. Although now widely available it is an expensive technique and may be difficult for young children unless sedated.

3.6.2 X-ray computed tomography (CT; Level IV)

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Figure 3.11. X-ray computed tomography.

X-ray computed tomography (CT) uses X-rays to differentiate between tissues and image quality is superior to MRI although the high radiation doses make it unsuitable in paediatric research.

3.6.3 Total body potassium (Level I)

Total body potassium scanning is based on measuring the emission of radioactive ^{40}K by the BCM. Scanning takes place with the subject supine and takes about 15 minutes and is a simple procedure although some subjects may experience claustrophobia. Information about BCM is helpful since it reflects better the functional component of weight but calculation of FFM is hampered by the uncertainty of the K component of FFM in children and a lack of reference data in the UK.

3.7 Summary

- Measurements of height and weight do not give any information about the components of body composition.
- The method of body composition analysis used will depend on availability, cost, environment and ease of use for measuring children.
- Accuracy and precision are important considerations for longitudinal analysis if true significant changes are to be identified.
- All techniques have limitations although the 4CM is considered a criterion method because assumptions are minimal;
 - Prediction techniques use regional or superficial proxies (sub-cutaneous SFT or resistance to an electrical current) and assume a relationship with body components (whole body FM and hydration of FFM) to predict FM or FFM. The value of predicted techniques is limited because; (i) they convert everyone to the average and (ii) are population specific (predominantly healthy).
 - 2CM utilise age and sex specific constant values for the nature of the FFM and therefore convert everyone to the average. Since these constants are derived from healthy populations the accuracy in patients who may have altered hydration or mineralisation may be reduced.
 - 3CM have greater accuracy than 2CM because they address the hydration of FFM, however, there is an assumption about the relationship between FM and MM which may be disrupted in illness.

Chapter 4. Hypotheses, recruitment, methods, ethical considerations and study plan

4.1 Hypotheses

Based on the issues highlighted in Chapter 2, I propose the following hypotheses to be tested in this thesis:

***Hypothesis 1:** There is no significant difference in body composition between children with CF and healthy children at baseline but differences will become apparent over time, with reductions in FM and FFM in CF patients.*

This hypothesis was tested by matching each 6-12 year old CF child with a healthy child of the same sex and ethnic background and within 1 year of age and performing 4CM body composition measurements to establish baseline data. Group comparison was also made between CF and healthy children at 2 years from baseline using all available control children.

The research and analyses addressing this hypothesis can be found in **Chapters 5, 6 and 7.**

***Hypothesis 2:** Specific components of body composition at baseline and changes over time are associated with spirometry in children with CF and predict clinical outcome.*

Since FEV₁ has been an outcome measure in many previous studies this was chosen as the primary clinical outcome measure. Furthermore, studies have shown a relationship between FEV₁ and mortality in patients with CF (39;113). In addition, genotype and pancreatic insufficiency was recorded. Physical activity was assessed by questionnaire given at the time of body composition measurements. A second measurement after 2 years allowed for the relationship between changes in body composition and change in spirometry to be investigated.

The research and analyses addressing this hypothesis can be found in **Chapters 5, 6 and 7.**

Hypothesis 3: *The conclusions are the same regardless of whether matched- pairs, group comparisons or comparison with reference data are used.*

To test this hypothesis I analysed the data using; (i) matched-pair controls at baseline and group comparisons at baseline and longitudinally and (ii) reference data derived from a large cohort of contemporary children aged 4 to 22 years.

The research and analyses addressing this hypothesis can be found in **Chapters 5, 6 and 7.**

Hypothesis 4: *The simple prediction techniques to assess body composition (BIA and SFT) and 2CM techniques (hydrometry and DXA) will not be accurate enough to identify depletion or change in body composition over time in this group of children with CF due to systematic and/or random bias.*

This hypothesis was tested by comparing SDS (derived from our previously measured large contemporary reference population) for FM assessed by DXA and SFT and FFM assessed by DXA, TBW and BIA with values from the 4CM.

The research and analyses addressing this hypothesis can be found in **Chapter 8.**

4.2 Aims

This thesis had 3 aims;

1. To investigate the effect of CF on body composition in young children with CF and whether this changes with growth and maturity. In addition, to investigate whether the type of comparison (pair-, group-match or compared to a reference population) affects the outcome of the analysis.
2. To investigate the relationship between body composition and clinical status assessed by FEV₁.
3. To investigate simple body composition techniques to define which would be most appropriate in clinical practice where 4CM is not available.

4.3 Recruitment and exclusion criteria

Children with CF aged 6 to 12 years under the care of Great Ormond Street Hospital NHS Trust, London were approached to take part in the study. The children with CF were diagnosed on the basis of confirmatory genetics or a positive sweat test with chloride values of > 60 mmol/L. Those recruited were clinically stable, that is to say free from lower respiratory tract infections and not requiring a change in antibiotics, steroids or bronchodilator treatment during the previous 14 days and emotionally able to undergo the measurements. Children with other conditions affecting body composition such as growth hormone therapy, diabetes and liver disease were excluded.

Healthy children for pair-matched, group-matched and cross-sectional reference comparison were recruited for another study of body composition at University College London, Institute of Child Health, London via schools and adverts in two London newspapers. The study started in February 2002 and those recruited were not premature (<36 weeks gestation), had a birth weight ≥ 2 kg and did not have any medical conditions or medication affecting body composition. The measurements were performed by myself using identical techniques and equipment. Measurements in 533, 4-22 year healthy subjects were used to generate SDS for SFT, BIA, TBW, 4C FM, 4C FFM, 4C PM, 4CMM, DXA FM and DXA FFM and 100 (6 – 12 years) of the original cohort were measured after 2 years for comparison with the children with CF. Measuring age-matched control children longitudinally allows adjustment for factors such as activity level and pubertal status and additionally has the benefit of comparing both groups of children longitudinally at similar points in time and not just as a cross-sectional comparison with a cohort who may or may not be contemporary.

4.4 Methods

4.4.1 Anthropometry

Body weight was measured as an integral stage of the Bodpod procedure to within 0.01kg with the child dressed in a swimsuit. Accuracy was confirmed by use of two solid weights of known mass. Standing height was measured to within 0.1cm with a wall-mounted digital display stadiometer (Holtain, Dyfed, United Kingdom). BMI was calculated as weight (kg) divided by the square of height (m). Waist, hip and MUAC were measured to 1 mm using a fibreglass tape and bicep, tricep, subscapular and supra-iliac SFT were taken in triplicate to nearest 2 mm and averaged. The waist was measured at the 'natural waist', half way between the 10th rib and iliac crest. Measurements were taken on the left side according to the method of Lohman et al (1988) using a Holtain skinfold calliper. Intra-operator technical error of measurement (TEM) was calculated on 50 children with CF and 50 control children using measurements made at the beginning of the study. The TEM varied according to the measurement type ranging from zero for weight, 0.2 mm (0.1%) for height and 0.8mm (10.6%) to 0.1mm (0.6%) for skinfold thickness depending on site.

4.4.2 Bio-electrical impedance

The Tanita Body Fat Analyzer (BC-418 MA), measures impedance using a constant current source (50kHz, 90μA) and 8 electrodes (2 on each foot and hand) which allows measurement of resistance in the whole body and segmentally (trunk, right and left arm and leg). A print-out of impedance and %fat, FM, FFM and TBW for these segments is obtained after the subject stands bare-foot on the foot plates and holds the hand grips for about one minute. Only impedance is measured, the other outcomes being calculated from equations in the machine's software. Only raw impedance from the print-out was used and converted to SDS (155) for the purposes of this thesis.

4.4.3 Dual-energy X-ray absorptiometry

Whole body bone mineral content (BMC), bone mineral density (BMD), FM and lean mass (non-osseous fat-free mass) was determined using a Lunar Prodigy whole-body fan beam scanner (GE Medical Systems, USA) in conjunction with software v.8.2 or 12.1. The scan was performed with the subject lying supine and took 5 to 10 minutes depending on height. In addition a scan of the LS (L2-L4) was performed with the subject supine and with the legs elevated on a soft padded block to derive LS BMC, BMD and bone area. The position of the legs aids contact of the lower spine with the scanner bed. All scans were performed with the subject wearing light indoor clothing and with no removable metal objects present. The scans are analysed automatically although manual changes by the operator may be made if the regions are inaccurate. The radiation exposure per whole-body scan is estimated to be 2 μ Sv, lower than daily background level. All scans were performed by one operator. Precision of bone density, established by repeat measurements of phantoms on 6 successive days, was <2%.

As mentioned previously, DXA has limitations when measuring soft tissue (102;196;197). However, soft tissue analysis by DXA is a readily available, practical alternative to more expensive methods such as CT and MRI. Regional soft tissue analysis (trunk, arms and legs) as well as whole body was therefore compared to 4CM in this thesis. Data from DXA was used for modelling the 4CM and for comparisons of simpler techniques compared to the 4CM in Chapter 8. In addition DXA FM was used as a covariate instead of 4C FM when investigating change in 4C FFM and DXA FFM used instead of 4C FFM for change in 4C FM, thereby using two independent outcomes in the analyses.

4.4.4 Deuterium oxide dilution

TBW was assessed by deuterium dilution, using a dose equivalent to 0.05 g deuterium per kg body weight (99.9 atom percent excess, Sigma Chemical Co. Chemicals, Poole, UK)). Doses were made up with water to approximately 100ml. Saliva samples were taken pre-dose and 4-5 hours post dose using absorbent

salivettes (Sarstedt, Rommelsdorf, Germany) at least 30 minutes after the last ingestion of food or drink. Samples were spun as soon as possible using a centrifuge and stored at -20°C until analysed by Iso-Analytical Ltd (Sandbach, UK) using the equilibration method of Scrimgeour (1993). Briefly, 0.3 ml of liquid, along with a vial of 5% platinum on alumina powder (Sigma-Aldrich, Poole, UK), was placed in a septum sealed container (Labco, High Wycombe, UK) and flushed for 2 minutes with hydrogen. Low enrichment and high enrichment standard waters were similarly prepared, in order to normalise data against Vienna Standard Mean Ocean Water standards. Samples equilibrated at room temperature for 3 days prior to analysis. The head spaces in the containers were then analysed for deuterium enrichment on a continuous flow isotope ratio mass spectrometer (Geo20-20, Europa Scientific, Crewe, UK). The accuracy of analyses was checked by measuring an intermediate water standard within each batch of samples. All samples were prepared and analysed in duplicate. The mean standard deviation of deuterium analyses by the equilibration technique in the laboratory is $< 2.5\%$.

Deuterium dilution space was assumed to overestimate TBW by a factor of 1.044 (180) and correction was made for fluid intake during the equilibrium period to derive actual body water. Data was used in modelling the 4CM and also in the comparison of 2CM techniques in Chapter 8. An example of the saliva collection information sheet may be found in **Appendix 6**.

4.4.5 Air-displacement plethysmography

Body volume was measured using air displacement plethysmography (ADP; Bodpod, Cosmed, Rome, Italy) whilst the subject wore a close fitting swimming costume and hat. The procedure (193) involves warming up the equipment by several 'autoruns' for 30 mins and then calibrating using a 50 litre metal cylinder. The subject sits in the anterior chamber which is connected to a posterior measurement chamber by oscillating diaphragms (which induce pressure change in the anterior chamber) whilst breathing normally. The procedure for one complete measurement is at least 2 measurements of body volume (taking 50 secs each) unless they differed by more than 150 mls in which case a 3rd volume measurement is

performed automatically by the machine. The raw volume values that appear transiently on the screen were recorded and to improve precision the whole procedure was repeated until two mean values for raw density of within 0.007kg/l were obtained (194). Where it was not possible to achieve two such measurements (possibly due to breathing irregularities in the CF children) the mean of all raw volume values was used after values $\pm 2SD$ were discarded (achieved over four separate tests of a minimum of 8 and maximum of 12 volumes). An adjustment of the mean raw volume was then made using predicted lung volume (189;201) and surface area (190) using children's equations to obtain the actual body volume. All measurements were made by one of two operators (JEW, CMW) and the data was used in the 4CM.

4.4.6 Spirometry

Laboratory spirometry, FEV_1 was measured by technicians in the lung function laboratory at Great Ormond Street Hospital NHS Trust according to protocols based on American Thoracic Society and European Respiratory Society standards for spirometry (202) adapted for children (203) using a Jaeger MasterScreen spirometer. Values for analysis were calculated as FEV_1 % and SDS compared to a large reference population (204). Children with FEV_1 % <45% were classified as having severe impairment, FEV_1 % ≥ 45 and <65% as moderate impairment, ≥ 65 % and <85% as mild impairment and ≥ 85 % as normal lung function.

4.5 Outcome measures used for calculation of body composition

Weight (kg)

Total body BMC (kg) from DXA

TBW (L) from isotope dilution

Body volume (L) from ADP

FM (kg) and FFM (kg) from DXA

Z (Ω) from BIA

SFT (mm)

4.5.1 Simple body composition techniques

4.5.1.1 Anthropometry

SDS for bicep, tricep, subscapular and supra-iliac SFT were calculated compared to our large contemporary population (4.6.2).

4.5.1.2 Bio-electrical impedance analysis

Whole body BIA was performed and height^2/Z calculated and converted to SDS compared to the reference population (4.6.2). Values of FM and FFM from the printout were not used due to the assumptions used in generation of the data which would be inappropriate for this population.

4.5.1.3 Dual-energy X-ray absorptiometry (DXA)

FM and lean mass values were obtained from a whole body DXA scan as described above and FFM was calculated as:

$$\text{FFM} = \text{lean mass} + \text{BMC}$$

Equation 4-1

Absolute values of FM and FFM and SDS (4.6.2) were used for whole body and regional (trunk, legs and arms) body measurements.

4.5.1.4 Deuterium oxide dilution

TBW may be used to calculate FFM, assuming an age and sex specific hydration factor of FFM, (H_{ffm}) according to published values (183;184).

$$\text{FFM} = \text{TBW} / H_{\text{ffm}}$$

Equation 4-2

FM was then calculated as;

$$\text{FM} = \text{weight} - \text{FFM}$$

Equation 4-3

And

$$\% \text{fat} = (\text{FM} / \text{weight}) \times 100$$

Equation 4-4

Weight, FM and FFM in kg, TBW in L

However, as discussed previously this method requires an assumption that the child has an average hydration. For the purposes of this thesis TBW SDS were also calculated for comparison of techniques.

4.5.1.5 Air-displacement plethysmography

Whole body density was calculated from body volume (which has been adjusted for residual lung volume and surface area artefact) and weight.

$$\text{Body density} = \text{weight} / \text{volume}$$

Equation 4-5

$$\% \text{ fat} = (527 / \text{body density}) - 485 \text{ (Wells 1999)}$$

Equation 4-6

And

$$\text{FM} = (\% \text{fat} \times \text{weight}) / 100$$

Equation 4-7

4.5.2 Four-component model

The 4CM uses values of BMC, weight, body volume and TBW to derive values for MM, TBW mass, FM and PM as described previously (167;199). Assumed densities of the four components were accounted for when calculating FM from the measurements;

$$\text{FM(kg)} = [(2.747 \times \text{volume}) - (0.710 \times \text{TBW})] + [(1.460 \times \text{BMC}) - (2.050 \times \text{weight})]$$

Equation 4-8

FFM was calculated as the difference between weight and FM:

$$\text{FFM} = \text{weight} - \text{FM}$$

Equation 4-9

$$\text{TBWmass} = \text{TBW volume} \times 0.99823$$

Equation 4-10

Hydration of the FFM (H_{FFM}) can be calculated as the ratio of TBWmass to FFM;

$$H_{\text{FFM}} = \text{TBWmass}/\text{FFM}$$

Equation 4-11

Mineral mass (MM) can be calculated as:

$$\text{MM} = \text{BMC} \times 1.2471,$$

this assumes a constant relationship between osseous to non-osseous mineral and is the only assumption in the 4CM

PM can then be calculated as;

$$\text{PM} = \text{Weight} - \text{FM} - \text{TBWmass} - \text{MM}$$

Equation 4-12

4.6 Assessment of confounding factors

Confounding factors were assessed by structured questionnaires.

4.6.1 Age

Age (years) was calculated as:

$$\text{Date of measurement} - \text{date of birth} / 365.25$$

Equation 4-13

4.6.2 Medical data

A brief medical history (**Appendix 5**) was obtained from the parent or guardian and included:

Serious childhood illness or surgery

Medication

Pancreatic status, genotype, presence of liver disease, diabetes, *Pseudomonas aeruginosa* and *Staphylococcus aureus* infection (in children with CF and confirmed with medical notes).

4.6.3 Physical activity

Physical activity level was assessed by asking the parent to give a rating of the child's activity as follows; 1) Much less than peers, 2) Less than peers, 3) Same as peers, 4) More than peers and 5) Much more than peers. This simple method has been shown to correlate well with physical fitness and BMI in children (205) although in a large survey of 13-14 year old children (206) it was fat deposition in girls and not BMI that was related to physical activity. Due to small numbers the lower two and upper two categories were combined resulting in three groups; less than, same as, and more than peers. In addition the number of hours spent in vigorous physical activity was recorded.

Examples of questionnaires and data collection sheets may be found in **Appendix 5** and saliva collection information in **Appendix 6**.

4.6.4 Pubertal status

Pubertal stage was self-assessed using line drawings showing the different Tanner stages for breast or genital development (**Appendix 8**). This avoids undressing and has been shown to be in good agreement with doctor assessment (207). For the purposes of analysis pre-pubertal (stage1) subjects were considered distinct from pubertal subjects (stages 2-5).

4.7 Statistical analyses

All analyses were performed using Statistical Package for Social Sciences 18.0 (SPSS Inc., Chicago) and $p < 0.05$ was considered significant.

4.7.1 Sample size

When deciding on the sample size required to show a difference between groups, type I and II error need to be considered. Type I error is the probability of rejecting the hypothesis when it is true and type II the probability of not rejecting the hypothesis when it is false (208). Therefore it is important to have sufficient numbers to address the research question confidently without subjecting more children than necessary to the research process.

Studies of children with CF have shown a positive relationship between BMI SDS and spirometry both cross-sectionally (52;72) and longitudinally (13;122) with Kastner-Cole et al noting an extra 10% of predicted FEV₁ for a 1 SDS higher BMI. The sample size was therefore calculated on the basis that a 0.5 difference in BMI SDS is likely to impact on clinical outcome and since BMI is a combination of FMI

and FFMI the calculated sample size should be sufficient to detect significant differences in both these tissues.

4.7.1.1 Matched pairs and longitudinal analysis

The conventional formula used to determine the number of children required to detect a difference between paired groups, and longitudinal comparisons where children are compared to baseline measurements is:

$$n = 8(sd^2/d^2)$$

Equation 4-14

where; n is the number per group, sd is the standard deviation, d is the difference between groups. This provides 80% power and a significance cut-off of 0.05 ($p < 0.05$). Using this formula the number of subjects needed to detect a given difference can be calculated or for a given SD of a trait, the magnitude and difference detectable for a given sample size. Therefore few subjects are needed to detect a large difference and more subjects are needed to detect a small difference. It would be useful to detect a small effect since; a) most biological factors create relatively small effects and b) previous research has not achieved a consensus on the critical values for healthy body composition in CF children. The difference (d) is expressed as SDS format which simplifies the formula to $n = 8/d^2$. Based on the above power calculations 8 subjects per group are sufficient to detect a difference of 1 SDS and 32 subjects per group to detect a difference of 0.5 SDS.

4.7.1.2 Cross-sectional group comparison

Although the intention was to continue with a matched-pair analysis at the 2 year measurement this was not possible due to either, not all the pairs being measured or having successful 4CM. Therefore, all available controls of the same age range as the children with CF were used. The formula to determine the number of children required to detect a difference with 80% power at 5% significance when using an independent t-test is:

$$n = 16(sd^2/d^2)$$

Equation 4-15

Based on the power calculation above, 16 subjects per group are sufficient to detect a difference of 1 SDS and 64 per group to detect a difference of 0.5 SDS.

This study aimed to recruit more than 64 (to maintain 64 throughout the study) in order to fulfill the sample size needed for between-group comparisons. Buntain et al. (36) quotes 23.5% of CF and 17% of controls dropping out over a 2 year study and Stettler et al. (96) reported a drop-out rate of 16% in CF children over 3 years. Since this study was constrained by time and available population (n = 116) all eligible children were approached for recruitment.

4.7.2 Size adjustment and calculation of standard deviation scores

Characteristics of all the children were compared to 1990 UK reference data (54;209) to generate SDS for weight, height, BMI and waist circumference using the lmsGrowth program© (210). A method analogous to that of BMI (weight/height²) was applied to absolute values of FM, FFM, PM and MM to remove the effect of height; FM index (FMI; FM/height²), FFM index (FFMI; FM/height²), PM index (PMI; PM/height²) and MM index (MMI; MM/height²) (211;212). The index 2, although appropriate to remove the relationship of height with FFM (including PM and MM) may not be the most appropriate for FM because FM is more variable and the index depends on age, sex and pubertal development. In patients, the appropriate index may be different from healthy people, for example, for this study at 6-12 years the index was, boys; CF 2.5, control 3.4 and girls; CF 2.9, control 2.4. At 8-14 years the index was all boys; 3.9 and girls; CF 4.6 and control girls 3. However, with our large reference population of 533 aged 4 to 22 years the index was 2.8. The problem of the appropriate index to use to adjust FM was addressed in a paper by Wells and Cole (211). They suggest 3 possible approaches; (1) where each group is of similar height the use of height² is appropriate, (2) where the groups are slightly different in height, check for real differences by regressing log_n height on log_n FM with group (patient v control) and height as independent variables and (3) where the height difference is great a log-log regression will give the power by which to raise height

in order to remove any effect of height on FMI. Wells and Cole suggest that approach 1 is valid because both groups are being compared with equal adjustment and in addition the error in FMI is considered trivial. They calculated that in the group of 8 year children studied, the variability in FMI due to height was 7.5%, much less than the variation due to fatness. I used approach (2) to investigate whether there were any differences in the slopes of data from CF children compared to controls in my cohort, and when log height was regressed on log FM I found that only log height was highly significant; group and group x log height interaction term were not significant. This suggests that the slope of each group does not differ significantly and approach 1 is valid. I have therefore used height² to adjust FM in this thesis, an index which is most likely to be used clinically given the difficulties of calculation and the variability of the appropriate index. The indices mentioned above were calculated using approach 3.

Using the lmsGrowth program© (210) the indexed variables were compared to measurements performed in a contemporary reference population of 533 healthy children aged 4 to 22 years (155) to produce SDS.

Total LS BMD SDS were generated from the Lunar Prodigy software using machine reference data matched for age, sex and ethnic group. BMD is a 2-dimensional measurement, which does not take into account bone size. Small children may have low BMC or BMD either because they have small bones and/or because they have less mineral than expected for the size of bone (213). To adjust for size the bone mineral apparent density (BMAD) a 3-dimensional ‘volumetric’ measurement was calculated for the LS as follows, from BMC and bone area (214).

$$\text{BMAD} = \text{BMC} / \text{bone area}^{1.5} \quad \text{Equation 4-16}$$

and then size adjusted SDS were calculated using UK reference data collected for the GE Lunar Prodigy (215).

4.7.3 Cross-sectional comparison of body composition variables

Using one-sided, independent and paired-sample *t*-tests comparison was made between; (i) both CF and control children and the 1990 UK reference population for anthropometric SDS, (ii) both CF and control children and the contemporary reference population for body composition variables SDS, (iii) between pair-matched (baseline) and group-matched (baseline and year 2) children with CF and healthy children for all anthropometry and body composition variables using absolute values and SDS. Initial analysis indicated a strong significant difference between the sexes and therefore they were analysed separately.

General linear models were used to examine the difference between children with CF and healthy controls taking into account factors predicting body composition variables, with matched-pair or group and puberty as fixed factors and age and height (for non-indexed variables) as continuous variables. Since total MM is predominantly bone mineral, MM was also adjusted for bone area to account for the effect of bone size as well as length (height). The same analysis was performed for baseline and 2 year measurements and may be found in Chapters 5 and 6.

4.7.4 Cross-sectional comparison of body composition and spirometry

Regression analysis was used to investigate the relationship between body composition variables and spirometry (FEV₁ % or FEV₁ SDS) in the children with CF. Height was included in the model rather than indexed body composition variables. The same analysis was performed for baseline and 2 year measurements and may be found in Chapters 5 and 6.

4.7.5 Longitudinal body composition and spirometry data analysis

To investigate the predictors of change in body composition variables over a 2 year period between measurements, it is necessary not only to consider the change but also the baseline value since the same absolute value of change will be very different for a child with optimum body composition compared to a child with suboptimal body composition. One possible way to account for this is to include both the baseline measurement and the change in the model. An alternative method uses the conditional change, that is, the amount of change once the effect of the baseline measurement has been removed. In this method the regression residuals (the values of the difference between the expected change and the actual change) are calculated for each child and these are the conditional change (216). The residuals have no relationship with the baseline measurement and therefore avoid auto-correlations. The expected change is calculated in the control boys and girls separately by regressing the baseline value on the 2 year value for each of the body composition variables. The regression equation is then used in the CF group to calculate the difference between actual change and the change expected if the child is growing the same as the healthy group (residual or conditional change). Where there is very little change from that which is expected, that is, when an individual is tracking the control group, absolute change and conditional change will be similar, as can be seen in the girls' graph (**Fig 4.1**) of absolute change compared to conditional change in FMI SDS. However, in the boys the differences are greater (**Fig 4.2**). Conditional values were therefore calculated for all the scale dependent variables and independent variables included in the regression.

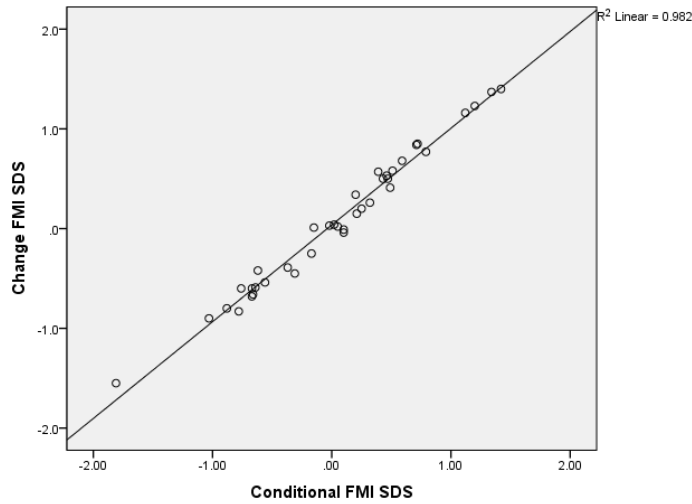


Figure 4.1. Correlation between absolute and conditional change in fat mass index ($FM/height^2$; FMI SDS) in girls with cystic fibrosis

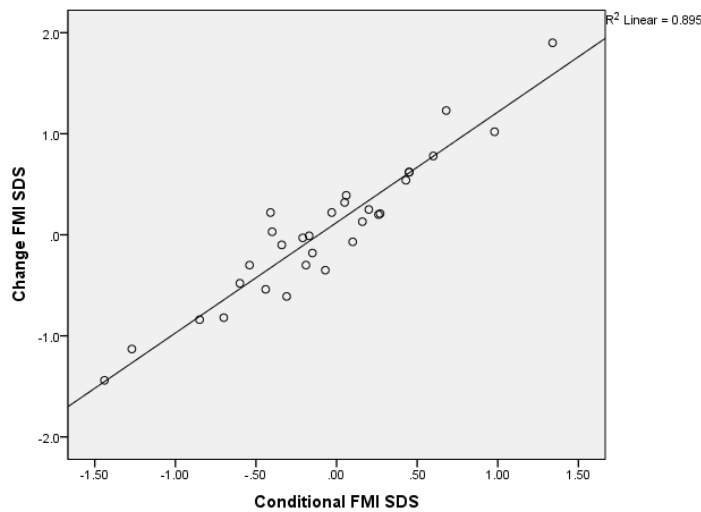


Figure 4.2. Correlation between absolute and conditional change in fat mass index ($FM/height^2$; FMI SDS) in boys with cystic fibrosis

Conditional change in SDS in those who had longitudinal measurements was calculated as change compared to the control boys or girls in this study (n = 95). SDS were calculated from a large reference population (n= 533) with only a single

measurement, therefore, conditional scores are a comparison within a smaller population.

Multiple regression analysis was used to investigate predictors of conditional change in either FMI SDS, FFMI SDS or MMI SDS in CF and control children separately and also between body composition variables and conditional change in FEV₁ SDS for children with CF. Choice of variables included in the models is discussed in **Chapter 7**.

Multiple regression analyses with the 2 year measurement as the dependent variable, the baseline as the covariate and condition (CF or control) as fixed factor were performed to discover any significant effects of having CF over the change in body composition variables. The analyses can be found in **Chapters 7**.

4.7.6 Cross-sectional comparison of simple techniques and the 4-component model

Data for males and females and for every available time-point (2001-2011) were pooled for purposes of the analyses. The accuracy of the simpler methods was assessed using the criterion 4CM as the reference method for adiposity (4C FM SDS) and leanness (4C FFM SDS). DXA FM, SFT and BMI SDS were used as simple measures of adiposity and DXA FFM, TBW and BIA (height^2/Z) SDS were used as simple measures of leanness.

SDS compared to our contemporary reference population (see 4.6.2) for body composition variables were calculated for absolute values of 4C FM and FFM, DXA FM and FFM (whole body and regional) TBW, BIA and SFT and BMI compared to the UK 1990 reference data (54;209). In addition DXA height adjusted values for FM and FFM (whole body and regional) were calculated as described in 4.6.2.

Significance of the mean value for all SDS from zero was tested using independent *t*-tests, and paired *t*- tests were used to compare the mean difference of the simpler methods with that of the 4CM.

The method of Bland and Altman (217) was used to assess agreement between the SDS for FM and FFM by the simpler techniques and the criterion 4CM. The mean difference between techniques (bias; simple technique-4CM) and the ± 2 SD of the difference between techniques (limits of agreement) were calculated. The bias was then tested for significance from zero by using a paired *t*- test. The extent to which the magnitude of the bias was related to the magnitude of the variable was calculated as the correlation between the difference and the mean of the measured values. Correlations were performed unadjusted, adjusted for age and adjusted for age and sex. This comparison is presented in **Chapter 8**.

Backward regression analyses were used to identify significant predictors of the bias in FM(I) and FFM(I) SDS. Analyses were performed with age, sex and BMI SDS as explanatory variables with sex coded as male = 1 and female = 2.

4.7.6.1 Categorisation of 'abnormal' standard deviation scores

A 'normal' body composition SDS was classed as an SDS between -2 and +2, the values typically used in clinical practice. The ability of each technique to categorise 'normal' and 'abnormal' body composition was assessed by cross-tabulation of the simple technique with the 4CM and calculation of Cohen's kappa coefficient (κ) and % agreement. Whilst % agreement is a measure in absolute terms, Cohen's kappa also takes into account the probability of agreement occurring by chance, a value of 1 indicating perfect agreement. This analysis is presented in Chapter 8.

4.7.7 Longitudinal comparison of simple techniques and the 4-component model

The ability of the simpler techniques to assess longitudinal change in non-indexed SDS was compared to change assessed by the 4CM. Two-year change in anthropometric and body composition SDS were compared to no change using

independent *t*- tests. Bland-Altman analyses were used to compare the bias in change (change by simple technique – change by 4CM) of body composition variables with limits of agreement of $\pm 2SD$. The degree to which the magnitude of the bias was related to the magnitude of the variable was assessed with correlation analyses (Chapter 8).

4.7.8 ‘Wisdom of crowds’ approach to determine whether aggregate predictions improve accuracy.

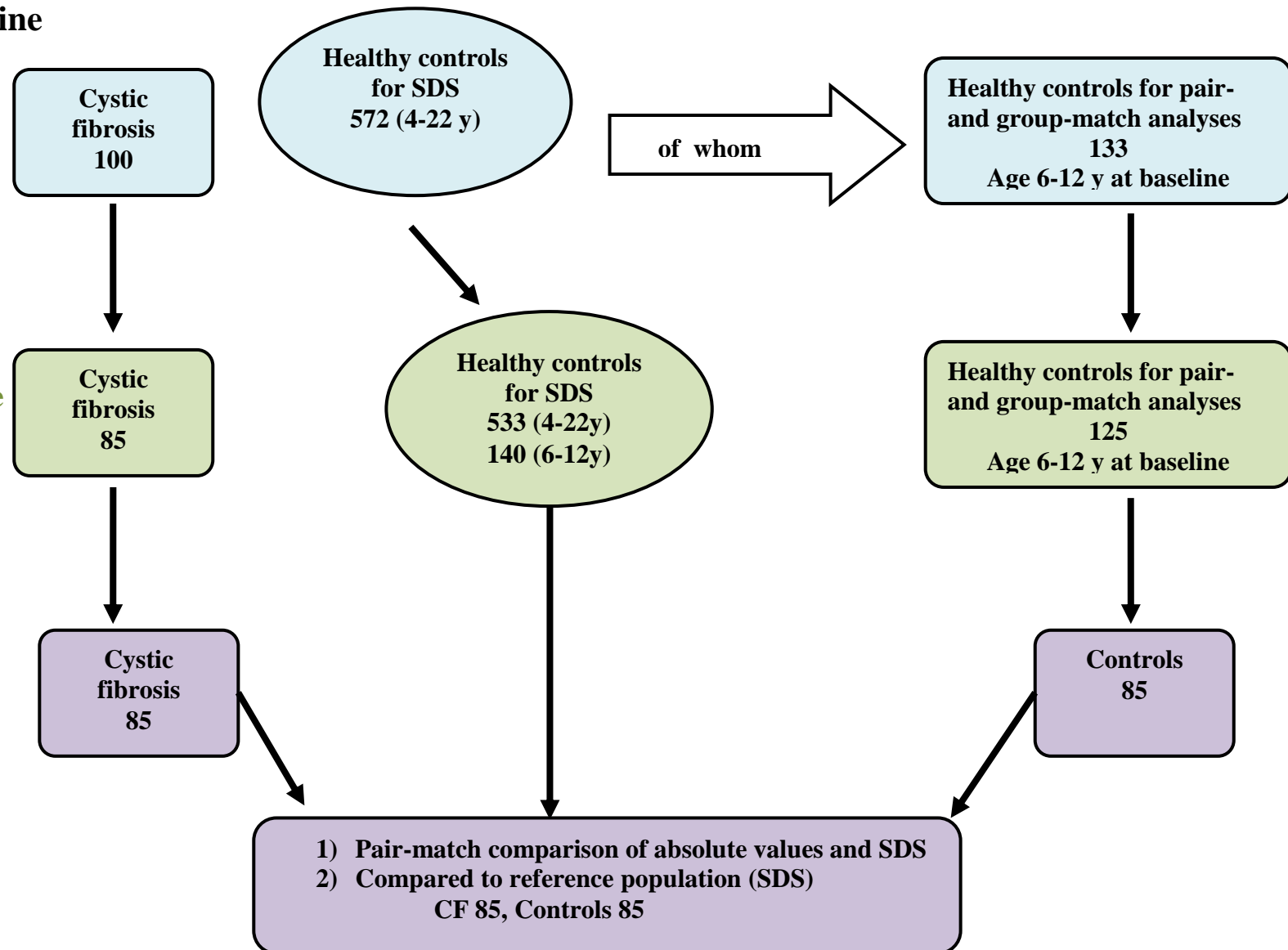
In a situation where the more sophisticated body composition techniques are not available it would be helpful to be able to use the simplest, readily available prediction techniques. Although the underlying assumptions may be flawed and the equations used to predict body composition may not be derived in the appropriate population, there is an approach which may allow such prediction techniques to be used. The ‘wisdom of crowds’ approach (218) is based on the theory that using many predictions will give an answer closer to the truth than using one or two predictions. In order to achieve improved accuracy four conditions must be satisfied: first, many predictions based on diverse criteria must be used; second, the predictions must be independent of each other; third, they must be based on different underlying assumptions; and fourth, the independent predictions must then be aggregated. The theory suggests that error will be randomly spread across the predictions and tend to cancel out, thereby improving the accuracy of the overall prediction. To test this hypothesis in this particular population I used 12 different prediction equations based on height, weight, SFT and BIA to calculate FM for each child and then aggregated them to compare with the 4CM (Chapter 8).

4.8 Study outline

Recruited

Successful
4CM at baseline

Chapter 5
Cross-sectional
at baseline



Study outline continued

**Successful
4CM at baseline**

Cystic fibrosis
85
Age 6-12 y at
baseline

Healthy controls
for SDS generation

533 (4-22 y)
140 (6-12 y)

Healthy controls for pair-
and group-match analyses
125
Age 6-12 y at baseline

**Successful 4CM
at baseline and
2 years**

Cystic
fibrosis
69

Controls
93

**Chapter 6, cross-sectional
group-match and compared to
reference population, baseline
and 2 years**

1. Group-match comparison of absolute values and SDS
2. Compared to reference population (SDS)
CF 69, Controls 93

**Chapter 7, longitudinal change
group-matched and reference
population**

4.9 Ethical considerations and study plan

Ethical permission was obtained from the ethical committee of the Institute of Child Health/ Great Ormond Street Hospital for Children NHS Trust. The project complied to the Data Protection Act 1998 and as such all measurement data was anonymised and hard copies of names and addresses were kept locked in a separate drawer from the measurement data sheets which were also kept locked. All electronic versions were password protected and saliva samples carried the same anonymised IDs.

Children with CF and their parent(s) were initially asked if they were interested in taking part by one of the doctors in the Respiratory Medicine department. If they agreed their contact details were given to me and I telephoned to give information and answer any questions. The appropriate information sheets were then sent by post or email and a follow-up call was made after at least 2 weeks to organise an appointment if they agreed and to answer any questions. Samples of information sheets and appointment letters can be found in **Appendices 2 & 3**.

Control children were recruited for an on-going study of the body composition of healthy children. Originally, ethical approval (**Appendix 1**) was for 1 measurement and subsequently those of the appropriate age range (6-12years) were approached for two yearly measurements under the ethics approval for the CF study. After a telephone call to ascertain acceptance, the appropriate information sheets were sent by post or email and 2 weeks later a telephone call was made to answer questions and arrange an appointment.

Measurements took place in the body composition suite sited in the radiology department of Great Ormond Street Hospital for Sick Children, taking on average 1hr 45mins. At the appointment, after explanation of the procedures, written consent was obtained from parents or young people over 16years, written assent was obtained from children aged 11-15 years and verbal assent was obtained from all the younger children. See **Appendix 4** for copies of parent, participant consent forms and child

assent forms. Travel expenses were reimbursed, and all participants received a print-out of their whole-body DXA scan, a certificate (**Appendix 7**) and £10 WH Smith voucher as a token of appreciation.

Chapter 5. Baseline body composition of 6-12 year old children with cystic fibrosis compared to healthy children and the relationship with lung function.

5.1 Introduction

Previous studies of growth and body composition have found that children with CF are often shorter and that there may be deficits of FM and FFM although some studies found the CF children had healthy body composition when young (see Chapter 2). In addition, the literature notes that FFM is positively related to lung function. However, there are many inconsistencies in the literature which may be partly due to the methodologies used. This study uses the more accurate ‘criterion’ 4CM in order to avoid some of the problems of the simpler methods. Data from simpler techniques is discussed in Chapter 8.

In this chapter I present a comparison of body composition between CF and healthy children using both pair-match and comparison with a reference population with the aim of testing hypothesis 1, that there will be no significant differences at baseline. In addition, examining associations between spirometry and body composition in children with CF to test hypothesis 2, (that specific components of body composition are associated with lung function).

5.2 Study design

This is a cross-sectional study comparing the body composition of 6-12 year children with CF to that of healthy children of the same age and sex and will form the baseline measurements for a subsequent measurement at 2 years. Comparisons were made firstly, with age (to within 1 year), sex and ethnicity matched healthy controls

and secondly, using SDS calculated from a larger contemporary population (see 4.6.2). In addition, the lung function, measured on the same day (or within 4 weeks if not possible) was related to components of body composition in the children with CF.

5.3 Recruitment and exclusion criteria

Children with CF were recruited from the patients at Great Ormond Street Hospital NHS Trust and the control children from an on-going study of healthy children at University College London, Institute of Child Health. Details can be found in Chapter 4.

5.4 Methods

Height, weight, waist, hip and MUAC and SFT were measured as described in Chapter 4. Body composition was assessed using the 4CM which uses whole body BMC from DXA, TBW from isotope dilution and density from ADP. LS and whole body BMC and BMD were measured by DXA to assess bone status. Confounding factors such as pubertal status and activity were assessed with questionnaires, the details of all these can be found in Chapter 4 and Appendices 5 and 6. Due to low numbers in some groups the classification of physical activity was recategorised in 3 groups; more than, same as and less than peers. Tanner staging was classified as pre-pubertal (stage 1) or pubertal (stages 2-5).

The children with CF also had lung function assessed; details can be found in Chapter 4.

5.5 Statistical analyses

5.5.1 Size adjustment and SDS calculation

Characteristics of all the children were compared to 1990 UK reference data to generate SDS for weight, height, BMI and waist circumference using the lmsGrowth program© (210). Size adjustment of body composition variables was made as described in Chapter 4. SDS were calculated compared to the contemporary reference population (see Chapter 4) for FM, FFM, PM, MM, and SFT. Total and LS BMD SDS were generated from the Lunar Prodigy software using machine reference data matched for age, sex and ethnic group and a size adjusted BMAD SDS was calculated as described in Chapter 4.

5.5.2 Comparison of body composition variables

Initial analysis indicated a strongly significant difference between the sexes and therefore they were analysed separately.

5.5.2.1 Compared to reference data

Using a one-sample *t*-test comparison was made between; (i) both CF and control children and the 1990 UK reference population for anthropometric SDS and (ii) both CF and control children and the contemporary reference population for body composition variables SDS.

5.5.2.2 Compared to pair-match

Paired sample *t*- tests were used to compare CF and healthy children for all anthropometry and body composition variables. General linear models were used to examine the difference between children with CF and healthy controls taking into account factors predicting body composition variables, with matched-pair, group and puberty as fixed factors and age and height (for non-indexed variables) as continuous

variables. Since total MM is predominantly bone mineral, MM was also adjusted for bone area to account for the effect of bone size as well as length (height).

5.5.3 Lung function

Simple regression analysis was used to investigate the relationship between body composition variables and spirometry (FEV₁ % and SDS) in the children with CF. Height was included in the model rather than using indexed body composition variables. MM was adjusted for LS bone area to account for the size of the bones.

5.6 Results

5.6.1 Subjects

100 of 116 eligible children with CF were recruited and complete 4CM measurements were obtained in 90 (4 children (3 girls) refused to enter the Bodpod, 6 post dose isotope dilution samples were inadequate volume for analysis (4 girls)). However, body water calculation was implausible for 5 children (3 girls), therefore pair-matching with healthy age, sex and ethnicity matched control children was made for 85 CF children ((boys; 37 (44%) and girls; 48 (56%)). 85 paired measurements should be sufficient to detect a 0.31 SDS difference in BMI and body composition variables between the groups with 80% power at 5% significance. Characteristics of the children are shown in **Table 5.1**. Self-assessed pubertal status for the 170 children was pre-pubertal for; boys (CF; 34 (92%), controls; 31 (71%) and girls (CF; 34 (71%), controls; 29 (60%). All subjects with CF except for 1 girl were pancreatic insufficient; had a wide range of pulmonary disease with a median FEV₁% 85.1%, with values between 32% and 131%; 8 (7 girls) had a gastrostomy in situ; 4 (3 girls) had liver disease and 1 girl was diabetic. Genotype was homozygous $\Delta F508$ for 59 children, heterozygous $\Delta F508$ for 19 children, 5 children had non $\Delta F508$ genotype and 2 children were of unknown genotype. There were 21 cases (10 boys) of chronic pseudomonas aeruginosa of the lungs and 15 cases (7 boys) with chronic

staphylococcus aureus infection. Sixteen eligible children with CF (9 girls) who were not recruited and 15 children (10 girls) who were measured and not included in the analysis were not significantly different with respect to height SDS, weight SDS and BMI SDS.

5.6.2 Anthropometry

5.6.2.1 Compared to UK reference data

Characteristics of the children are shown in Table 5.1. The healthy children were representative of the 1990 UK reference data apart from the girls being significantly heavier ($p<0.01$) and both boys and girls having greater waist circumference ($p<0.01$). Boys with CF were significantly shorter ($p<0.05$) but had a higher BMI SDS ($p<0.01$) and waist circumference ($p<0.01$) and girls with CF were lighter ($p<0.001$), shorter ($p<0.001$), had lower BMI SDS ($p<0.05$) but also had greater waist circumference ($p<0.01$) than the 1990 UK reference data.

5.6.2.2 Compared to pair match

Comparison between children with CF and healthy pair-matched controls indicated that the boys with CF were not significantly different apart from higher BMI SDS ($p<0.01$) and waist circumference SDS ($p<0.05$). The range of BMI SDS was; boys with CF; -1.5 to 2.4 and control boys; -1.9 to 2.0. The girls with CF were significantly shorter ($p<0.01$) and lighter ($p<0.01$) with lower anthropometric values for all measures apart from waist circumference (non-significant).

5.6.3 Body composition

Comparison by reference populations and case-control can be found in **Table 5.2** with a summary in **Table 5.3**.

5.6.3.1 Compared to reference data

Comparison of CF children with a reference population (Lunar Prodigy reference data for bone and reference data for body composition (155) indicated a higher total BMD SDS ($p<0.001$) and FFMI SDS ($p<0.01$) in boys with CF and lower LS BMD SDS ($p<0.001$), BMAD SDS (size adjusted; $p<0.01$), FMI SDS ($p<0.001$), FFMI SDS ($p<0.05$) and MMI SDS ($p<0.001$) in girls.

5.6.3.2 Compared to pair-match

Boys with CF had significantly higher total BMD SDS ($p<0.05$) and FFMI SDS ($p<0.01$) compared to case control boys. Girls with CF had less total BMD SDS ($p<0.05$) and LS BMD SDS ($p<0.001$) and significantly lower FMI SDS ($p<0.001$) and MMI SDS ($p<0.001$).

Table 5.1. Characteristics of cystic fibrosis (CF) and control children in pair-match analyses (P_{group}) and compared to reference data (P_{ref}).

	CF boys (n=37)				CF girls (n=48)				Control boys (n=37)			Control girls (n=48)		
	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}	Mean	SD	P_{ref}
Age (y)	8.9	1.17	NA	0.336	9.6	1.62	NA	0.570	9.0	1.21	NA	9.6	1.78	NA
Weight (kg)	29.5	6.35	NA	0.518	30.0	7.19	NA	<0.001	28.8	6.68	NA	34.6	9.36	NA
Height (m)	1.30	0.08	NA	0.068	1.32	0.11	NA	<0.001	1.33	0.10	NA	1.37	0.11	NA
BMI (kg/m ²)	17.3	2.10	NA	0.009	16.4	1.99	NA	0.001	16.1	1.76	NA	18.1	2.84	NA
Weight SDS	0.11	1.04	0.505	0.338	-0.56	1.04	0.001	<0.001	-0.11	0.91	0.456	0.42	0.98	0.005
Height SDS	-0.41	1.01	0.017	0.118	-0.59	1.12	0.001	0.001	-0.05	0.95	0.740	0.22	0.87	0.084
BMI SDS	0.50	0.95	0.003	0.005	-0.33	0.95	0.020	0.001	-0.11	0.87	0.454	0.39	1.10	0.019
Waist (cm)	63.1	5.92	NA	0.001	60.2	5.5	NA	0.071	58.1	4.41	NA	62.2	7.67	NA
Waist SDS	1.24	0.79	<0.001	0.001	0.69	0.77	<0.001	0.161	0.34	0.70	0.005	0.94	1.05	<0.001
Hip (cm)	67.7	6.17	NA	0.876	68.2	7.06	NA	<0.001	67.9	6.70	NA	74.6	8.63	NA
MUAC (cm)	19.8	2.39	NA	0.996	19.7	2.60	NA	<0.001	19.8	2.74	NA	22.2	3.31	NA
Sum 4 skinfold thickness (mm)	34.7	19.8	NA	0.236	37.1	14.6	NA	0.007	29.08	11.7	NA	48.4	21.1	NA
4 skinfold thickness SDS	0.14	0.95	0.379	0.185	-0.41	0.83	0.001	0.005	-0.14	0.76	0.270	0.14	0.96	0.326
Pre-pubertal (n,%)	34	92	NA	NA	34	71	NA	NA	31	84	NA	29	60	NA
FEV ₁ %	91.3	20.9		NA	77.6	18.4	NA	NA			NA			NA
FEV ₁ SDS	-0.70	1.66	0.017	NA	-1.89	1.56	<0.001	NA			NA			NA
Pancreatic insufficient (n)	37		NA	NA	47		NA	NA			NA			NA
Liver disease (cases)	1		NA	NA	1		NA	NA			NA			NA
Diabetes (cases)	0		NA	NA	1		NA	NA			NA			NA
Ps aerug (cases)	10		NA	NA	11		NA	NA			NA			NA
Staph aureus (cases)	7		NA	NA	8		NA	NA			NA			NA

BMI; body mass index (weight/height²), SDS; standard deviation score compared to UK 1990 reference data (54;209), MUAC; mid-upper arm circumference, Sum 4 skinfold thickness; bicep+tricep+subscapular+ suprailiac, n=79, P_{group} paired *t*-test, CF boys and Control boys or CF girls and Control girls; P_{ref} ; one sample *t*-test CF and Controls compared to UK 1990 reference data. FEV₁%; forced expired volume in 1s percentage of the expected, FEV₁ SDS; compared to reference data (204)). Pre- puberty is based on Tanner staging 1 (pre) to stages 2 – 5 (pubertal), Pancreatic insufficient; reduced pancreatic function requiring the oral addition of pancreatic enzymes, Ps aerug; chronic pseudomonas aeruginosa infection of the lungs, Staph aureus; chronic staphylococcus aureus infection of the lungs.

Table 5.2. Body composition of cystic fibrosis (CF) and control children used in pair-matched analyses (P_{group}) and compared to reference data (P_{ref}).

	CF boys (n=37)				CF girls (n=48)				Control boys (n=37)			Control girls (n=48)		
	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}	Mean	SD	P_{ref}
Body volume (L)	28.3	6.4	NA	0.500	27.8	7.09	NA	<0.001	27.5	6.51	NA	33.6	9.41	NA
Total body water (L)	18.0	3.1	NA	0.872	17.1	3.72	NA	0.001	17.9	3.71	NA	18.9	4.31	NA
Total bone mineral content (kg)	1.05	0.2	NA	0.606	1.01	0.24	NA	<0.001	1.07	0.24	NA	1.20	0.33	NA
Total bone mineral density (g/cm ²)	0.90	0.0	NA	0.020	0.87	0.05	NA	0.009	0.88	0.05	NA	0.90	0.07	NA
Total bone mineral density SDS	0.58	0.6	<0.001	0.011	-0.11	0.74	0.329	0.028	0.23	0.50	0.007	0.23	0.62	0.012
LS bone mineral density (g/cm ²)	0.73	0.0	NA	0.358	0.73	0.10	NA	0.026	0.71	0.08	NA	0.78	0.11	NA
LS bone mineral density SDS	-0.03	0.7	0.795	0.340	-0.49	1.12	0.004	0.055	-0.19	0.75	0.129	-0.05	0.93	0.688
LS bone mineral apparent density SDS	0.18	0.9	0.267	0.149	-0.40	1.19	0.024	0.424	-0.12	0.85	0.413	-0.20	1.06	0.203
4-Component model														
Fat mass (kg)	5.95	3.66	NA	0.413	6.13	2.93	NA	<0.001	5.31	2.80	NA	9.46	4.71	NA
Fat mass index SDS	0.02	1.01	0.907	0.325	-0.76	0.89	<0.001	0.157	-0.22	0.97	0.166	0.02	0.95	0.861
Fat (%)	19.0	7.84	NA	0.501	20.4	5.47	NA	<0.001	17.8	6.14	NA	26.1	7.46	NA
Fat-free mass (kg)	23.5	3.60	NA	0.976	22.7	4.98	NA	0.001	23.5	5.09	NA	25.1	5.60	NA
Fat-free mass index SDS	0.56	1.01	0.002	0.003	-0.39	1.07	0.016	0.157	-0.14	1.04	0.414	-0.08	0.94	0.557
Fat-free mass hydration (%)	75.9	2.07	NA	0.740	74.9	1.97	NA	0.427	76.0	1.49	NA	75.1	1.54	NA
Fat-free mass density (kg/L)	1.087	0.007	NA	1.000	1.090	0.007	NA	0.317	1.087	0.005	NA	1.091	0.005	NA
Protein (kg)	4.31	0.65	NA	0.955	4.46	1.06	NA	0.112	4.30	1.18	NA	4.72	0.99	NA
Protein mass index SDS	0.36	1.36	0.113	0.103	0.08	1.03	0.585	0.528	-0.16	1.11	0.376	-0.05	0.97	0.731
Mineral mass (kg)	1.34	0.28	NA	0.637	1.28	0.28	NA	<0.001	1.36	0.30	NA	1.52	0.41	NA
Mineral mas index SDS	0.07	0.84	0.601	0.095	-0.84	1.10	<0.001	0.001	-0.23	0.87	0.11	-0.04	0.92	0.792

SDS; standard deviation score, LS ; Lumbar spine (L2-L4) bone mineral apparent density which is a 3-D ‘volumetric’ measurement that is calculated from the 2-D Bone Mineral Density (BMD) actually measured by the DXA machine, SDS are calculated from reference data (215). Fat mass index (fat mass/height²), Fat-free mass index (fat-free mass/height²), Protein mass index (protein mass/height²), Mineral mass index (fat mass/height²). SDS are calculated from reference data collected in 533 contemporary healthy children (155). P_{group} paired t -test, CF boys and Control boys or CF girls and Control girls; P_{ref} one sample t - test CF and Controls compared to reference data

Table 5.3. Summary of comparisons (before adjustment for age, height and puberty)

	Boys with CF compared to;		Girls with CF compared to;	
	Pair-match	Reference	Pair-match	Reference
Weight SDS	NS	NS	↓	↓
Height SDS	NS	↓	↓	↓
BMI SDS	↑	↑	↓	↓
WAIST SDS	↑	↑	NS	↑
FMI SDS	NS	NS	↓	↓
FFMI SDS	↑	↑	NS	↓
PMI SDS	NS	NS	NS	NS
MMI SDS	NS	NS	↓	↓

Paired *t*-test with pair-match and one sample *t*-test compared to zero for reference data. Reference data is UK 1990 (54;209) for weight, height, BMI and waist and reference data from 533 children measured by the 4-component model (155). FMI; fat mass index (FM/height²), FFMI; fat-free mass index (FFM/height²), PMI; protein mass index (PM/height²), MMI; mineral mass index (MM/height²).

5.6.3.3 *Potential confounders affecting body composition outcomes*

Table 5.4 (boys) and **Table 5.5** (girls) show adjusted mean differences in body composition (CF - control) before and after adjustment for height and puberty. Boys with CF had a greater weight ($p<0.001$) and waist circumference ($p<0.001$) SDS than controls. In addition, FFMI SDS was significantly higher ($p<0.01$) in boys with CF although the absolute FFM adjusted for actual height was not significantly different. Girls with CF had lower weight ($p<0.05$), height ($p<0.001$), FM ($p<0.01$), hip and MUAC ($p<0.01$) than control girls. In addition, MMI SDS ($p<0.01$) but not absolute MM, was significantly less in CF girls. An analysis of only pre-pubertal girls ($n=24$ pairs) confirmed the deficit in FM. Additional adjustment of MM for bone area (to adjust for bone size as well as length) did not affect the outcome in either sex. To determine whether differences in body composition were due to different levels of activity the parent's rating of the child's activity level was added to the model with no effect on the outcome (data not shown).

5.6.3.4 *Hidden depletion of fat-free mass*

Two girls in this study had BMI SDS in the normal range (> -1.64 SDS (5th centile) and <1.64) with FFMI SDS below -1.64 (5th centile). Girl A, aged 8 had a BMI SDS of -0.82 and FFMI SDS -4.60 and girl B, aged 7 had a BMI SDS of -1.37 and FFMI SDS of -2.53 .

5.6.3.5 *High BMI*

Four boys with CF had a BMI SDS >1.64 (95th centile). BMI/FMI SDS for these boys were: (a) $1.71/1.49$, (b) $1.83/1.49$, (c) $1.96/0.32$ and (d) $2.42/1.99$.

5.6.4 Relationship between body composition and spirometry

The mean (SD) FEV₁ SDS for 83 patients (1 boy and 1 girl did not perform spirometry) was; boys -0.70 (1.66) $p<0.05$ and girls -1.89 (1.56) $p<0.001$. A regression analysis of factors associated with FEV₁ SDS is presented in **Table 5.6**. FM SDS ($p<0.01$) and BMI SDS ($p<0.05$) were significantly positively associated with FEV₁ SDS in girls only. A similar model with either FEV₁ or FEV₁ % indicated a similar pattern. A plot of FEV₁% and FMI SDS for girls is shown in **Figure 5.1**

indicating a positive relationship ($r=0.40$, $p=0.005$) however, four girls had a high FEV₁% with a low FMI SDS.

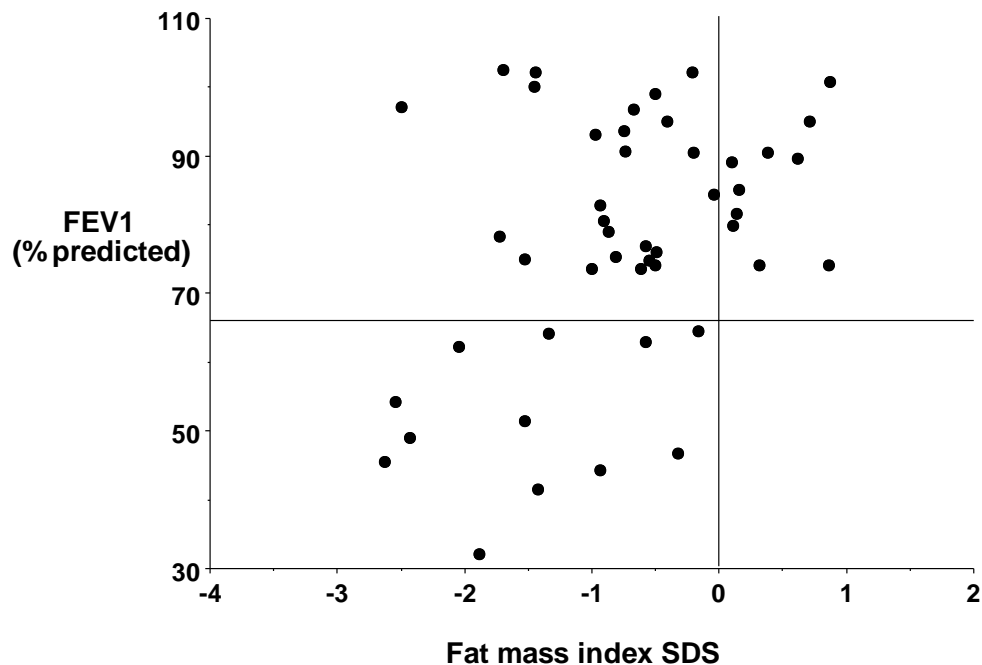


Figure 5.1. Relationship between percent predicted forced expiratory volume in 1 s (FEV₁) and fat mass index (FMI; $FM/height^2$) standard deviation scores in girls with cystic fibrosis assessed by the 4-component model of body composition. Correlation in 47 girls with cystic fibrosis; $r = 0.40$, $p = 0.005$

5.6.5 Body composition assessed by simple techniques

Data for body composition assessed by simple techniques (SFT, BIA, TBW and DXA) compared to the 4CM is presented in Chapter 8.

Table 5.4. Difference in size and whole-body composition between boys with cystic fibrosis and control boys (CF minus control, n=37 matched pairs). Positive mean values indicate a higher value in the CF group compared to controls.

	Adjusted for age			Adjusted for age and height			Adjusted for age, height and puberty		
	Mean	SEM	P	Mean	SEM	P	Mean	SD	P
Weight (kg)	0.65	1.10	0.556	2.04	0.74	0.009	2.13	0.82	0.010
Weight SDS	0.18	0.24	0.442	0.51	0.14	0.001	0.51	0.14	0.001
Height (m) ¹	-0.02	0.01	0.109				-0.01	0.01	0.284
Height SDS ¹	0.36	0.23	0.129				-0.20	0.20	0.328
BMI (kg/m ²) ¹	1.01	0.40	0.017				1.13	0.41	0.009
BMI SDS ¹	0.55	0.20	0.009				0.61	0.20	0.004
Total body water (L)	0.07	0.45	0.882	0.65	0.29	0.034	0.66	0.30	0.033
Body volume (L)	0.73	1.14	0.527	2.12	0.79	0.011	2.13	0.81	0.013
Total body density (kg/L)	-0.002	0.004	0.598	-0.004	0.004	0.299	-0.004	0.004	0.328
Fat mass (kg)	0.66	0.79	0.412	1.29	0.73	0.084	1.28	0.74	0.093
Fat mass index (kg/m ²) ¹	0.47	0.40	0.24				0.55	0.41	0.184
Fat mass index SDS ¹	0.24	0.25	0.351				0.30	0.26	0.250
Fat-free mass (kg)	-0.05	0.62	0.933	0.71	0.43	0.111	0.72	0.44	0.106
Fat-free mass index SDS ¹	0.60	0.20	0.005				0.64	0.21	0.004
Fat-free mass hydration (%)	-0.01	0.46	0.983	-0.02	0.48	0.969	-0.03	0.49	0.941
Fat-free mass density (kg/L)	0.000	0.001	0.770	0.000	0.002	0.837	0.000	0.002	0.862

General linear model, adjusting for group, matched pairs and puberty as fixed factors and age and height as continuous variables. SDS; standard deviation score, BMI; body mass index (weight/height²), ¹ adjusted for age and puberty only. **Table continues on next page**

Table 5.4 continued. Difference in size and whole-body composition between boys with cystic fibrosis and control boys (CF minus control, n=37 matched pairs). Positive mean values indicate a higher value in the CF group compared to controls.

	Adjusted for age			Adjusted for age and height			Adjusted for age, height and puberty		
	Mean	SEM	P	Mean	SEM	P	Mean	SD	P
Protein mass (kg)	-0.04	0.19	0.817	0.09	0.18	0.619	0.10	0.18	0.597
Protein mass index SDS ¹	0.38	0.29	0.189				0.42	0.29	0.161
Mineral mass (kg)	-0.02	0.04	0.599	0.03	0.02	0.225	0.03	0.02	0.218
Mineral mass adjusted (kg) ²	-0.002	0.03	0.940	0.03	0.02	0.239	0.03	0.02	0.238
Mineral mass index SDS ¹	0.23	0.16	0.173				0.25	0.17	0.140
LS bone mineral density (g/cm ²)	0.01	0.02	0.499	0.02	0.02	0.340	0.02	0.02	0.307
LS bone mineral density SDS	0.10	0.16	0.525	0.17	0.16	0.308	0.17	0.16	0.299
LS bone mineral apparent density SDS	0.26	0.20	0.204	0.21	0.21	0.336	0.21	0.21	0.330
Waist circumference (cm)	4.85	1.02	<0.001	5.76	0.90	<0.001	5.76	0.92	<0.001
Waist circumference SDS	0.87	0.18	<0.001	1.05	0.14	<0.001	1.05	0.15	<0.001
Hip circumference (cm)	-0.18	1.22	0.883	1.22	0.92	0.196	1.20	0.94	0.209
Mid-upper arm circumference (cm)	-0.11	0.54	0.842	0.28	0.51	0.590	0.28	0.52	0.591
Log 4 skinfold thickness ³	0.04	0.12	0.753	0.05	0.13	0.678	0.06	0.13	0.643
4 skinfold thickness SDS ⁴	0.29	0.14	0.049	0.43	0.20	0.042	0.43	0.21	0.045

General linear model, adjusting for group, matched pairs and puberty as fixed factors and age and height as continuous variables. SDS; standard deviation score, LS; lumbar spine bone mineral apparent density (size adjusted bone mineral density) SDS compared to Lunar Prodigy software; anthropometric SDS compared to UK 1990 data (54;209), body composition and 4 skinfold SDS compared to contemporary reference data (155). ¹ Adjusted for age and puberty only, ² Adjusted for LS bone area, ³ Log 4 skinfold thicknesses (bicep, tricep, subscapular and supra-iliac) n=36 pairs, ⁴ Mean of bicep, tricep, subscapular and supra-iliac SDS n= 36 pairs

Table 5.5. Difference in size and whole-body composition between girls with cystic fibrosis and control girls (CF minus control, n=48 matched pairs). Positive mean values indicate a higher value in the CF group compared to controls.

	Adjusted for age			Adjusted for age and height			Adjusted for age, height and puberty		
	Mean	SEM	P	Mean	SEM	P	Mean	SD	P
Weight (kg)	-5.71	1.31	<0.001	-1.95	1.02	0.063	-1.99	0.87	0.027
Weight SDS	-1.01	0.22	<0.001	-0.35	0.15	0.029	-0.35	0.14	0.014
Height (m) ¹	-0.05	0.01	<0.001				-0.05	0.01	0.001
Height SDS ¹	-0.36	0.23	0.232				-0.73	0.21	0.001
BMI (kg/m ²) ¹	-1.76	0.48	0.001				-1.35	0.39	0.001
BMI SDS ¹	-0.75	0.20	0.001				-0.58	0.17	0.001
Total body water (L)	-1.88	0.55	0.001	-0.02	0.30	0.937	-0.04	0.27	0.890
Body volume (L)	-5.85	1.34	<0.001	-2.16	1.09	0.053	-2.21	0.93	0.022
Total body density (kg/L)	0.01	0.003	0.001	0.009	0.004	0.015	0.009	0.003	0.008
Fat mass (kg)	-3.39	0.75	<0.001	-2.11	0.78	0.009	-2.14	0.69	0.003
Fat mass index (kg/m ²) ¹	-1.53	0.35	<0.001				-1.26	0.30	<0.001
Fat mass index SDS ¹	-0.81	0.19	<0.001				-0.69	0.17	<0.001
Fat-free mass (kg)	-2.46	0.73	0.002	0.03	0.40	0.939	0.02	0.37	0.965
Fat-free mass index SDS ¹	-0.34	0.21	0.118				-0.22	0.20	0.284
Fat-free mass hydration (%)	-0.28	0.35	0.427	-0.56	0.40	0.172	-0.57	0.38	0.146
Fat-free mass density (kg/L)	-0.001	0.001	0.329	0.000	0.001	0.934	0.000	0.001	0.911

General linear model, adjusting for group, matched pairs and puberty as fixed factors and age and height as continuous variables. SDS; standard deviation score, BMI; body mass index (weight/height²), ¹ adjusted for age and puberty only. **Table continues on next page**

Table 5.5. continued. Difference in size and whole-body composition between girls with cystic fibrosis and control girls (CF minus control, n=48 matched pairs). Positive mean values indicate a higher value in the CF group compared to controls.

	Adjusted for age			Adjusted for age and height			Adjusted for age, height and puberty		
	Mean	SEM	P	Mean	SEM	P	Mean	SD	P
Protein mass (kg)	-0.26	0.16	0.113	0.23	0.18	0.055	0.23	0.12	0.058
Protein mass index SDS ¹	0.12	0.21	0.567				0.15	0.21	0.475
Mineral mass (kg)	-0.25	0.06	<0.001	-0.07	0.04	0.063	-0.07	0.04	0.057
Mineral mass adjusted (kg) ²	-0.11	0.05	0.018	-0.07	0.038	0.074	-0.07	0.04	0.067
Mineral mass index SDS ¹	-0.83	0.22	0.001				-0.71	0.21	0.002
LS bone mineral density (g/cm ³)	-0.05	0.02	0.028	-0.004	0.02	0.847	-0.01	0.02	0.829
LS bone mineral density SDS	-0.45	0.22	0.050	0.01	0.22	0.977	0.002	0.22	0.992
LS bone mineral apparent density SDS	-0.21	0.26	0.429	0.08	0.29	0.773	0.08	0.28	0.784
Waist circumference (cm)	-2.25	1.08	0.042	-0.33	1.11	0.771	-0.38	0.90	0.675
Waist circumference SDS	-0.29	0.16	0.084	0.04	0.16	0.820	0.03	0.14	0.829
Hip circumference (cm)	-6.39	1.31	<0.001	-3.00	1.13	0.011	-3.05	0.96	0.003
Mid-upper arm circumference (cm)	-2.55	0.56	<0.001	-1.36	0.54	0.015	-1.38	0.48	0.007
Log 4 skinfold thickness ³	-0.24	0.09	0.009	-0.11	0.10	0.289	-0.15	0.09	0.124
4 skinfold thickness SDS ⁴	-0.18	0.10	0.082	-0.31	0.21	0.143	-0.36	0.20	0.074

General linear model, adjusting for group, matched pairs and puberty as fixed factors and age and height as continuous variables. SDS; standard deviation score, LS; lumbar spine bone mineral apparent density (size adjusted bone mineral density) SDS compared to Lunar Prodigy software; anthropometric SDS compared to UK 1990 data (54;209), body composition and 4 skinfold SDSs compared to contemporary reference data (155). ¹ Adjusted for age and puberty only, ² Adjusted for LS bone area, ³ Log 4 skinfold thicknesses (bicep, tricep, subscapular and supra-iliac) n=43 pairs, ⁴ Mean of bicep, tricep, subscapular and supra-iliac SDS n= 43 pairs

Table 5.6. Simple regression analysis of factors (adjusted for height)¹ associated with forced expired volume in 1s standard deviation scores

	Boys (n=37)					Girls (n=48)				
	B	SEM	<i>t</i>	<i>P</i>	<i>r</i> ²	B	SEM	<i>t</i>	<i>P</i>	<i>r</i> ²
BMI SDS	0.41	0.29	1.40	0.171	0.026	0.52	0.23	2.23	0.031	0.079
Fat mass (kg)	0.10	0.10	1.00	0.324	0.030	0.26	0.09	2.82	0.007	0.124
Fat-free mass (kg)	-0.03	0.18	-0.15	0.880	0.059	0.15	0.13	1.14	0.261	0.005
Mineral mass (kg) ²	0.02	0.15	0.14	0.892	0.090	-0.05	0.15	-0.34	0.734	0.032
DXA bone mineral content (kg) ²	0.65	3.20	0.20	0.841	0.090	4.17	2.44	1.77	0.094	0.010
DXA bone mineral density (g/m ³)	-4.14	6.01	-0.69	0.495	0.044	4.52	5.67	0.80	0.430	-0.019

SDS; standard deviation score, DXA; dual-energy X-ray absorptiometry, ¹except BMI SDS, ² additionally adjusted for lumbar spine bone area

5.7 Discussion

In this chapter I; (i) compared the body composition of children with CF to healthy children using both case control and reference populations and (ii) investigated associations between body composition and lung function in children with CF.

5.7.1 Body composition

Hypothesis 1 cannot be confirmed as this study identified clear sex differences in body composition in these young patients, indicating that abnormalities in females may be established much earlier than previously considered (59). Although boys with CF had body composition similar to healthy controls, girls had a deficit of FM. It is possible that the method we used to categorise pubertal development may not be sensitive to early hormonal changes; however, the deficit was seen in pre-pubertal girls. In accordance with previous research, children with CF in this study were shorter than healthy children but, surprisingly, boys had higher BMI SDS than healthy control boys and the reference population. This was mainly due to 4 boys with BMI SDS > 1.64 (95th centile) and the BMI/FMI SDS ratio for these boys show that high BMI SDS does not always reflect excess fat. Simple SFT measurement identified the boy with high FFM and low FM as having less subcutaneous fat than the other 3 boys. It may be prudent to identify the nature of high BMI in these patients and implement dietary interventions to avoid the complications of excess fat in adult life. There were 2 girls who had a BMI SDS in the normal range but very low (5th centile) FFMI SDS termed ‘hidden depletion of lean mass’ which has previously been reported in adults (104;117) and in children (105;150). The prevalence in the 2 studies of children were 54% (105) and 14% (150), much higher than this study however, the age range was much wider and included young adults and the criteria for normal weight/BMI and FFM varied. Girls with CF were smaller overall than the control girls, perhaps related to the slightly higher than expected weight of control girls and the lower proportion of pubertal girls and sub-optimal health (indicated by poorer lung function) of girls with CF.

I found that the control children had mean waist circumferences significantly greater than the UK reference data. For the girls this was not surprising given that they had a mean BMI SDS of 0.39 but the boys BMI SDS was -0.11. One explanation might be that the reference data was collected in 1988 and that the current obesogenic environment has produced a population of children with higher waist circumference SDS. Mc Carthy et al (161) who produced the waist circumference SDS used in this analysis acknowledged the need for more up-to-date data. The fact that SFT SDS was not high in the control group is likely to be due to the different populations measured for the reference data, 8,500 children for waist circumference in 1988 and 533 children for SFT from 2002 – 2007. However, the boys with CF had even higher waist SDS than the controls (1.24 ± 0.79) and the girls with CF had high waist SDS relative to their smaller size (0.69 ± 0.77) and this is in spite of the fact that FM SDS is not significantly different in the boys (CF- control; 0.30 ± 0.26) and girls with CF having significantly *less* FM (CF-control; -0.69 ± 0.17). One explanation might be that a distended abdomen commonly seen in children with CF is due to inflammation and enlargement of the internal organs rather than FM. This issue will be addressed in subsequent analyses in the following chapters by comparing regional soft tissue from DXA.

Body composition is influenced by many factors such as genotype, nutrition and activity. It is likely that nutritional intake impacts greatly on body composition although for the purposes of this thesis it was not possible to acquire accurate records of dietary intake and therefore the contribution of diet on body composition has not been included. The body composition of children with CF may be additionally affected by the inflammatory response to chronic bacterial infection leading to altered protein-energy balance, lack of appetite and reduced activity. 25% of children on this study had chronic infection by *pseudomonas aeruginosa* and exacerbations of inflammatory conditions are likely to impact negatively on nutritional status. Unfortunately it was not possible to acquire accurate data on inflammatory episodes in the group of children.

Our group previously reported normal BMD and BMAD in 32 8-12 yr old children with CF (99); although boys in the present study had normal BMD and BMAD the value for girls was significantly lower. The 24 pre-pubertal girls had normal bone

BMAD but low BMD ($p < 0.001$) suggesting low BMD reflects small size and possible delayed puberty.

Table 5.3 summarises the differences seen when comparing data from patients with those from either matched controls or a large reference population, and highlights some of the difficulties of comparing studies of different design. In this study, boys were identified as shorter compared to the reference population but not by case-control analysis, while girls had significantly lower FFMI SDS compared to the reference population but not to matched controls. Previous studies have not been consistent in accounting for height and puberty and tables 5.4 and 5.5 illustrate the different findings depending on which factors are accounted for in the analysis. The method of matching and analysis must be considered when comparing studies.

Tables 5.4 and 5.5 also give an indication of the differences depending on whether raw values are used as opposed to height adjusted SDS. For example, the girls with CF have less mineral than control girls and this is non-significant when using the value of MM but significant when using MMI SDS. This may be in some part due to the value of the index used to adjust for height being 2 rather than the ideal index to remove the effect of height which, in this study is 2.1 for CF and 1.6 for control girls. In addition, there was a difference in outcome depending on comparison using FFMI SDS or FFM adjusted for age, puberty and height in boys. This may explain some of the variability in findings of previous studies.

Whereas body composition variables adjusted for height and converted to SDS are useful to monitor an individual's progress they may not be appropriate in group comparisons where adjusting for the individuals' *actual* height is an easy alternative.

5.7.2 Spirometry

I chose FEV₁ as an outcome measure because it is a good surrogate marker of clinical status and predicts mortality, and I found clear sex differences in the relationship between FEV₁ and body composition. Contrary to previous research in adults (117;118) and children (11;64;65), FFM was not associated with FEV₁ in either boys or girls but I identified a significant positive relationship with FM adjusted for height

in girls only. This discordance with previous studies may be due to the methodologies used, the narrow age range of young children studied, adjustment of FM for stature or analysing the sexes separately. The sex differences may reflect differences in body composition during growth, with relatively greater gains in FFM in boys and FM in girls. It is likely that the positive association between FM and lung function in girls is a reflection of their poor nutritional status, given the generally low FM in this group. However, some girls with low FM had good lung function (Figure 5.1); of the 4 girls with FMI SDS <-1.5 and $FEV_1 > 78\%$, 3 were more physically active than their peer group and 1 the same. This finding warrants further investigation and suggests that nutritional and physical therapy perhaps needs to be sex specific. It was not possible to analyse the data by genotype due to low numbers in each group.

5.8 Study limitations

I used predicted rather than measured lung volume when calculating body volume by ADP due to the difficulty of performing the technique for young children. However, a study of children with CF deriving FFM from ADP concluded that there was no significant difference in FFM calculations between measured or predicted lung volumes (219).

Since exercise will affect the amount of lean tissue we repeated the analyses taking into account parental reported exercise level; there was no difference in outcome. However, the parental rating may be affected by differing expectations in healthy and chronically ill patients and between the sexes and future studies should address this by using objective measures of physical activity.

This study indicates that the children with CF have larger waist circumferences than their healthy counterparts and for girls with CF this is despite having less FM than the control girls. Regional measures of FM may indicate whether the reduction in whole body FM is generalised throughout the body or restricted to regions. This issue is addressed in Chapter 6 where whole body and regional DXA soft tissue data is presented.

5.9 Summary

- This is the first study to use a ‘criterion’ method to measure body composition in children with CF.
- My findings do not support Hypothesis 1, that there will be no differences at baseline in young children with CF. I found clear sex differences even in pre-pubertal children, girls with CF showing deficits of FM and to a lesser degree MM and boys with CF having body composition similar to controls. Given the poorer prognosis in girls this warrants further investigation.
- Four boys with CF had a BMI SDS in the obese range, and in 3 cases this was due to excess fat. It would seem prudent to carefully monitor children with high BMI to avoid future health problems due to excess fat.
- Two girls with CF had depleted FFM with normal BMI highlighting the inability of height and weight measurement to monitor body composition.
- Contrary to previous research I found that FM related to FEV₁ in girls only.

Chapter 6. Cross-sectional comparison of the body composition of children with cystic fibrosis with that of healthy children at 6-12 and 8-14 years and the relationship with lung function.

6.1 Introduction

This chapter presents a cross sectional comparison between children with CF and control children at age 6-12 years and 8-14 years and includes only those children in whom there were two consecutive (2 years apart), successful 4CM measurements thereby insuring that comparison at baseline and 2 years is made in the same group of children. The analysis is (i) compared to reference populations and (ii) compared to group-matched (sex and age) and the relationship to the analysis in the previous chapter is shown in **Figure 6.1**. A study outline is shown in Chapter 4 (4.5).

<u>Chapter 5</u>	<u>Chapter 6</u>
Cross-sectional, compared to reference data and pair-matched analyses at baseline	Cross-sectional, compared to reference data and group-matched analyses at baseline and 2y
85 CF, 85 Control	69 of 85 CF, 72 of 85 Control + 21

Figure 6.1. *Description of mode of analysis and subjects in Chapters 5 and 6.*

Puberty is the time of greatest changes in body composition under the influence of the sex hormones with relatively greater gains in FFM in boys and FM in girls. Sub-optimal nutrition at this time is likely to impact negatively on growth and body composition.

The aims of this chapter are to test the hypotheses that; (1) there is no significant difference in body composition at baseline but differences will become apparent over time, (2) that specific components of body composition are associated with lung function in children with CF and hypothesis (3) that the conclusions are the same regardless of the mode of analysis, that is whether by comparison with reference population or by matching with a smaller control group.

6.2 Study design

This is a cross-sectional study comparing the body composition of children with CF and controls at age 6-12 years and two years later. Comparisons were made firstly, using SDS calculated from a large reference population (see 4.6.2) and secondly, with sex and age-matched (6-12 years at baseline) control children. In addition, the lung function, measured on the same day (or within 4 weeks if not possible) was related to components of body composition in the children with CF. Longitudinal analysis is presented in Chapter 7.

6.3 Recruitment and exclusion criteria

Children with CF were recruited from patients at Great Ormond Street Hospital for Children, the control children from an on-going study of healthy children at University College London, Institute of Child Health. Details can be found in Chapter 4.

6.4 Methods

Height, weight, waist, hip, MUAC and SFT were measured as described in Chapter 4. Body composition was assessed using the 4CM which uses whole body BMC from DXA, TBW from isotope dilution and density from ADP. Simpler techniques (SFT, BIA, DXA and TBW) are compared in Chapter 8. LS and whole body BMC and BMD

were measured by DXA to assess bone status and DXA FM and FFM (lean + bone) whole body and regional ((trunk and limbs (arms and legs)) to compare tissue distribution. Confounding factors such as pubertal status and activity were assessed with questionnaires, the details of all these can be found in Chapter 4 and Appendices 8 and 5. Due to low numbers in some groups the classification of physical activity was recategorised in 3 groups; more than, same as and less than peers. Tanner staging was classified as pre-pubertal (stage 1) or pubertal (stages 2-5). The children with CF also had lung function assessed, described in Chapter 4.

6.5 Statistical analyses

6.5.1 Size adjustment and standard deviation score calculation

Characteristics of all the children were compared to 1990 UK reference data (54;209) to generate SDS for weight, height, BMI and waist circumference. Size adjustment of body composition variables was made as described in Chapter 4. SDS were calculated compared to a contemporary reference population (see Chapter 4) for FM, FFM, PM, MM and SFT (155). Total and LS BMD SDS were generated from the Lunar Prodigy software using machine reference data matched for age, sex and ethnic group and a size adjusted BMAD SDS (215) was calculated as described in Chapter 4.

6.5.2 Comparison of body composition variables

Initial analysis indicated a strongly significant difference between the sexes and therefore they were analysed separately.

6.5.2.1 Compared to reference data

Separate comparisons at baseline and 2 years were made using a one-sample *t*-test between; (i) both CF and control children and the 1990 UK reference population for

anthropometric SDS and (ii) both CF and control children and the contemporary reference population for body composition variables and SFT.

6.5.2.2 Compared to control group

Comparison between CF and control children was made at baseline and 2 years; (i) using independent sample *t*- tests and (ii) using general linear models taking into account factors predicting body composition variables, with group and puberty as fixed factors and age and height (for non-indexed variables) as continuous variables. Since total MM is predominantly bone mineral, MM was also adjusted for bone area to account for the effect of bone size as well as length (height). Given the findings in chapter 5 that both control children and children with CF had higher mean waist circumference than expected, partial correlations were performed to investigate the proportion of variability in waist circumference that can be attributed to body composition. The relationship between waist circumference and FM, FFM, age, sex and height were calculated individually whilst adjusting for all the other factors. In addition regression analysis was performed with the same factors to determine the overall contribution to variability in waist circumference.

6.5.3 Spirometry

Simple regression analysis was used to investigate the relationship between body composition variables and FEV₁ SDS at baseline and 2 years. Height was included in the model rather than using indexed body composition variables.

6.6 Results

6.6.1 Subjects

Of the 100 children measured at baseline there were 16 children who did not have a second measurement; 6 left the care of Great Ormond Street Hospital (5 boys), 7

declined to be re-measured (2 boys) and 3 did not have time at their annual clinic visit (2 boys). There were no significant differences in respect of height, weight, BMI and FEV₁ between drop-outs and those remaining in the study (**Appendix 9**).

Of the 84 left in the study 15 were not used in the second analysis; 9 had failed 4CM at baseline, (2 refused complete 4CM measures (1 boy), 5 failed TBW (2boys), 2 failed ADP (1 boy)) and 6 had failed 4CM at 2years, (2 no saliva sample, 4 failed TBW (all girls)). There were no significant differences in the girls remaining in the analysis compared to those who were not included but the boys remaining in the analysis were significantly lighter with a lower BMI (**Table 6.1**).

Table 6.1. Children with cystic fibrosis not included in this analysis compared to those remaining

	Removed from baseline analysis						Remaining in analysis			
	Boys n= 6			Girls n=10			Boys n= 31		Girls n=38	
	Mean	SD	P	Mean	SD	P	Mean	SD	Mean	SD
Age	8.64	1.24	0.537	8.94	0.93	0.321	8.97	1.16	9.67	1.72
Height SDS	0.07	0.89	0.202	-0.59	1.09	0.854	-0.51	1.02	-0.59	1.12
Weight SDS	1.20	0.76	0.004	-0.74	0.96	0.849	-0.10	0.96	-0.51	1.11
BMI SDS	1.59	0.60	0.001	-0.64	1.47	0.570	0.29	0.86	-0.26	0.93
FEV ₁ SDS	-0.61	3.21	0.193	-1.97	1.69	0.693	-1.10	1.54	-1.56	1.44

Of the original 85 control children whose data was used in the previous chapter, 72 had valid 4CM measurements at baseline and 2 years. Pair-matching with the same pairs as the previous chapter would reduce the numbers for analysis considerably and therefore, in order to include as many subjects as possible, all available age appropriate healthy controls with valid measurements at both time-points were included in a group-match analysis. There was therefore an additional 21 control children included. Age, height, weight and BMI SDS did not differ between the control children at baseline in pair-match (Chapters 5) and group-match (Chapter 6) analyses (**Table 6.2**).

Table 6.2. Comparison of control children at baseline in Chapters 5 and 6.

	Chapter 5 analysis N = 85			Chapter 6 analysis N=93	
	Mean	SD	P	Mean	SD
Age	9.31	1.58	0.471	9.49	1.74
Height SDS	0.10	0.91	0.617	0.17	0.83
Weight SDS	0.19	0.98	0.878	0.17	0.88
BMI SDS	0.17	1.03	0.578	0.09	0.94

The analysis therefore was performed on data from 69 of the original 85 children with CF (31 boys and 38 girls) compared to 72 original and 21 additional control children (44 boys and 49 girls) matched for age. A sample size of 69 would be sufficient to detect a difference of 0.48 SDS in a group comparison with 80% power at $p=0.05$.

At baseline 80% of CF and 66% of control children were pre-pubertal and at the 2 year measurement 45% of CF and 30% of controls were pre-pubertal. For CF children genotype was homozygous $\Delta F508$ for 48 children, heterozygous $\Delta F508$ for 17 children, 3 children had non $\Delta F508$ genotype and 1 child was of unknown genotype. All subjects with CF except 1 girl were pancreatic insufficient, had a wide range of pulmonary disease with a baseline mean FEV₁% of 83.2%, with values between 33% and 123%, 7 (6 girls) had a gastrostomy in situ, 1 girl had liver disease and 1 girl was diabetic. At the 2-year measurement mean FEV₁% was 79.4%, with values between 32% and 119%, 8 (5 girls) had a gastrostomy in situ, 7 (4 girls) had liver disease and 3 girls were diabetic. At baseline 14 children (8 boys) had chronic infection of pseudomonas aeruginosa and 11 (6 boys) had chronic infection of staphylococcus aureus. The status of 2 girls was unknown. After 2 years 24 (15 boys) had pseudomonas aeruginosa and 14 (6 boys) had chronic staphylococcus aureus.

6.6.2 Anthropometry

Characteristics of all the children at each time point are presented in **Table 6.3** for boys and **Table 6.4** for girls.

6.6.2.1 Compared to reference data

At baseline and 2 years the control boys were not significantly different from the reference population in respect of height, weight, BMI and SFT SDS but had significantly larger waist SDS ($p < 0.01$). The control girls were significantly heavier and taller ($p \leq 0.01$) with larger waist circumference ($p < 0.001$) at baseline and 2 years. Boys with CF were significantly shorter than the reference data but the deficit although significant ($p < 0.05$) was less at the 2 year measurement and the mean waist circumference SDS was greater at both time points ($p < 0.001$) despite SFT SDS being average. CF girls differed more, being lighter and shorter at baseline ($p < 0.01$) and 2 year measurement ($p < 0.05$) with larger waist circumference SDS ($p < 0.001$) at both time points and SFT SDS below zero (non-significant).

6.6.2.2 Compared to control group

Boys with CF were shorter than controls with larger waist circumference at both time points ($p < 0.05$). At the 2 year measurement the boys with CF had significantly smaller hip and mid-upper arm circumferences ($p < 0.05$). Girls with CF had significantly lower weight and height ($p < 0.001$) and BMI SDS, hip and MUAC ($p < 0.05$) at both measurements. Despite being smaller than the control girls, the girls with CF had similar waist circumference at both time-points.

Regression analyses and partial correlations of the relationship between waist circumference and age, height, sex, FM and FFM may be found in **Appendix 10**. FM, FFM, age, sex and height explained 86% of the variation in waist circumference in children with CF and 91% in control children. Adjusted partial correlations can be found in **Figure 6.2**.

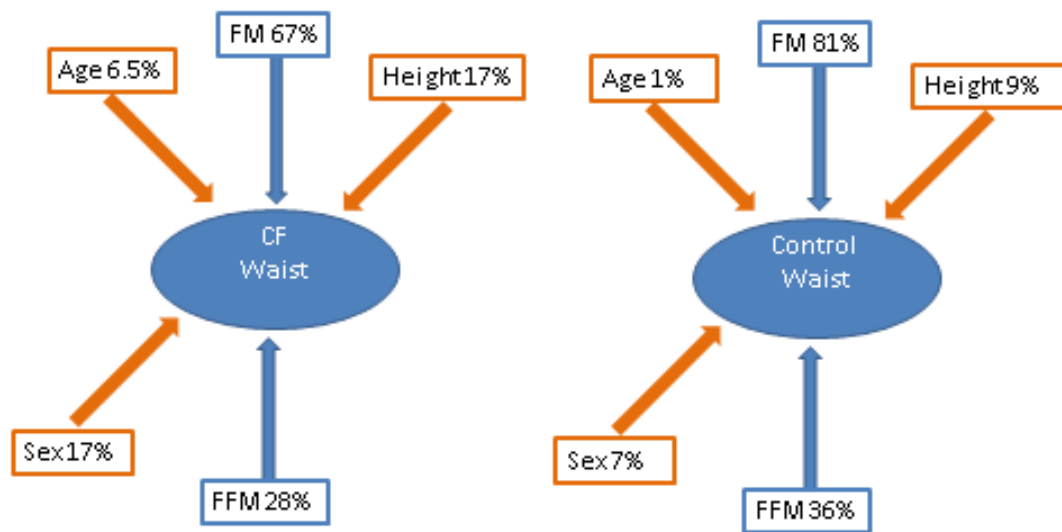


Figure 6.2. Contribution of body composition, age, sex and height to variability in waist circumference in children with cystic fibrosis and controls. Determined by partial correlations, each factor adjusted for the others, % is adjusted R^2

6.6.3 Body Composition

A comparison of body composition at baseline and 2 years is presented in **Table 6.5** (boys) and **Table 6.6** (girls) with a summary in **Table 6.8**.

6.6.3.1 Compared to reference data

Control boys did not differ from the cross sectional reference data in respect of FMI, FFMI, PMI and MMI SDS, however, they had significantly higher mean total BMD SDS at baseline ($p < 0.001$) but lower total and LS BMD at 2 years ($p < 0.05$). Size adjusted LS BMAD was not significantly different from zero at both time-points. Control girls were typical of the reference population apart from a higher mean total

BMD SDS at baseline ($p<0.01$). Boys with CF had higher mean total BMD SDS ($p<0.001$) at both time-points and higher FFMI SDS at baseline ($p<0.05$) whereas the girls with CF had lower LS BMD and BMAD, FMI, FFMI and MMI SDS ($p<0.05$) at both measurements.

Table 6.3. Characteristics of cystic fibrosis (CF) and control boys at baseline and two years with group comparison (P_{group}) and compared to reference data (P_{ref}).

	Baseline							Two years						
	CF n=31				Control n=44			CF n= 31				Control n=44		
	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}
Age (y)	8.97	1.16		0.108	9.50	1.36		11.0	1.19		0.151	11.5	1.36	
Weight (kg)	28.7	6.13		0.244	30.8	6.87		35.1	8.98		0.097	38.7	9.46	
Height (m)	1.30	0.09		0.010	1.36	0.08		1.41	0.10		0.011	1.47	0.10	
BMI (kg/m ²)	16.8	1.81		0.250	16.3	1.72		17.5	2.03		0.883	17.6	2.41	
Weight SDS	-0.10	0.96	0.579	0.826	-0.05	0.87	0.702	-0.13	0.98	0.454	0.337	0.09	1.00	0.548
Height SDS	-0.51	1.02	0.009	0.019	0.03	0.88	0.806	-0.42	1.02	0.032	0.019	0.13	0.91	0.366
BMI SDS	0.29	0.86	0.071	0.058	-0.09	0.84	0.469	0.15	0.94	0.388	0.561	0.01	1.06	0.951
Waist (cm)	62.1	5.43		0.016	59.1	4.88		65.6	5.60		0.214	63.8	6.35	
Waist SDS	1.07	0.70	<0.001	<0.001	0.35	0.73	0.002	0.88	0.70	<0.001	0.021	0.41	0.83	0.002
Hip (cm)	66.9	5.78		0.120	69.2	6.62		72.7	6.40		0.040	76.3	7.95	
MUAC (cm)	19.40	2.26		0.162	20.3	2.74		20.6	2.53		0.043	22.0	3.07	
Sum 4 skinfold thickness (mm) ¹	30.2	12.7		0.985	30.1	11.6		33.4	14.4		0.187	38.7	18.9	
4 skinfold thickness SDS ¹	-0.10	0.85	0.534	0.971	-0.09	0.79	0.448	0.001	0.85	0.997	0.331	0.20	0.89	0.142
FEV ₁ %	86.6	18.8						86.4	18.3					
FEV ₁ SDS	-1.10	1.54	<0.001					-1.15	1.56	<0.001				
Activity (hours per week)	9.39	6.95		0.340	8.05	5.14		9.19	5.18		0.767	8.84	4.94	
Pre-pubertal (n, %)	29	94%			31	71%		21	68%			14	32%	

P_{ref} ; one-sample t - test compared to zero, CF or control compared to reference data (54;155;209). P_{group} ; independent sample t -test, CF boys v control boys or CF girls v control girls, BMI; body mass index (weight/height²), SDS; standard deviation score compared to UK 1990 reference data (54;209), MUAC; mid-upper arm circumference, Sum 4 skinfold thickness; bicep+tricep+subscapular+ suprailiac, ¹CF n=30, control n= 44, FEV₁%; forced expired volume in 1s percentage of the expected, FEV₁ SDS; compared to reference data (204), Pre-puberty is based on Tanner staging 1 (pre) to stages 2 – 5 (pubertal), Pancreatic insufficient; reduced pancreatic function requiring the oral addition of pancreatic enzymes, Ps aerug; chronic pseudomonas aeruginosa infection of the lungs, Staph aureus; chronic staphylococcus aureus infection of the lungs.

Table 6.4. Characteristics of cystic fibrosis (CF) and control girls at baseline and two years with group comparison (P_{group}) and compared to reference data (P_{ref}).

	Baseline							Two years						
	CF n=38				Control n=49			CF n= 38				Control n=49		
	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}
Age (y)	9.64	1.76		0.757	9.67	2.03		11.07	1.80		0.666	11.6	2.04	
Weight (kg)	29.7	7.90		0.027	34.0	9.57		37.7	10.7		0.035	42.6	10.7	
Height (m)	1.33	0.11		0.078	1.38	0.13		1.44	0.12		0.061	1.49	0.12	
BMI (kg/m ²)	16.5	1.97		0.024	17.7	2.44		17.7	2.76		0.051	18.9	2.71	
Weight SDS	-0.51	1.11	0.007	<0.001	0.36	0.84	0.004	-0.52	1.30	0.017	<0.001	0.39	0.79	0.001
Height SDS	-0.59	1.12	0.003	<0.001	0.29	0.77	0.010	-0.53	1.18	0.017	0.001	0.39	0.74	0.004
BMI SDS	-0.26	0.93	0.089	0.016	0.25	1.00	0.085	-0.32	1.12	0.091	0.011	0.27	0.97	0.104
Waist (cm)	60.6	5.67		0.719	61.1	6.93		65.6	7.25		0.903	65.4	6.70	
Waist SDS	0.72	0.77	<0.001	0.657	0.80	0.93	<0.001	0.96	0.91	<0.001	0.858	0.99	0.85	<0.001
Hip (cm)	69.0	7.72		0.007	73.9	8.74		76.2	9.68		0.019	81.1	9.29	
MUAC (cm)	20.0	2.75		0.006	21.8	3.29		21.50	3.24		0.007	23.4	3.09	
Sum 4 skinfold thickness (mm) ¹	38.0	13.4		0.045	45.3	18.2		48.8	21.6		0.225	54.6	21.9	
4 skinfold thickness SDS ¹	-0.35	0.80	0.011	0.074	-0.01	0.92	0.081	-0.14	0.96	0.384	0.157	0.16	0.94	0.251
FEV ₁ %	80.3	18.1						74.8	19.1					
FEV ₁ SDS	-1.56	1.44	<0.001					-2.05	1.58	<0.001				
Activity (hours per week)	8.13	4.65		0.957	8.08	3.89		6.76	3.47		0.099	8.02	3.50	
Pre-pubertal (n, %)	26	68%			30	61%		10	26%			14	29%	

P_{ref} ; one-sample *t*-test compared to zero, CF or control compared to reference data (54;209). P_{group} ; independent sample *t*-test, CF boys v control boys or CF girls v control girls, BMI; body mass index (weight/height²), SDS; standard deviation score compared to UK 1990 reference data (54;209), MUAC; mid-upper arm circumference, Sum 4 skinfold thickness; bicep+tricep+subscapular+ suprailiac, ¹CF n=30, control n= 44, FEV₁%; forced expired volume in 1s percentage of the expected, FEV₁ SDS; compared to reference data (204), Pre- puberty is based on Tanner staging 1 (pre) to stages 2 – 5 (pubertal), Pancreatic insufficient; reduced pancreatic function requiring the oral addition of pancreatic enzymes, Ps aerug; chronic pseudomonas aeruginosa infection of the lungs, Staph aureus; chronic staphylococcus aureus infection of the lungs.

Table 6.5. Body composition of cystic fibrosis (CF) and control boys with group comparison (P_{group}) and compared to reference data (P_{ref}).

	Baseline							Two years						
	CF n=31				Control n=44			CF n= 31				Control n=44		
	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}
Body volume (L)	27.4	6.15		0.250	29.1	6.72		33.6	7.93		0.062	37.7	9.85	
Total body water (L)	17.7	3.13		0.261	18.6	3.58		21.2	4.26		0.149	22.9	5.20	
Total bone mineral content (kg)	1.05	0.23		0.108	1.14	0.24		1.32	0.37		0.114	1.45	0.33	
Total bone mineral density (g/cm^2)	0.90	0.06		0.475	0.89	0.06		0.95	0.08		0.001	0.79	0.11	
Total bone mineral density SDS	0.53	0.62	0.001	0.096	0.30	0.50	0.001	0.57	0.76	0.001	0.001	-0.27	0.82	0.033
LS bone mineral density (g/cm^2)	0.73	0.08		0.652	0.73	0.08		0.78	0.10		0.565	0.79	0.11	
LS bone mineral density SDS	-0.09	0.68	0.467	0.687	-0.16	0.76	0.173	-0.19	0.73	0.154	0.611	-0.29	0.84	0.029
LS bone mineral apparent density SDS	0.07	0.85	0.656	0.951	0.06	0.90	0.683	0.17	0.93	0.327	0.456	-0.01	1.07	0.938
4-Component model														
Fat mass (kg)	5.25	2.98		0.381	5.88	3.11		7.02	3.35		0.242	8.15	4.55	
Fat mass index SDS	-0.21	0.99	0.242	0.968	-0.20	0.94	0.159	-0.17	0.85	0.286	0.757	-0.10	0.77	0.504
Fat (%)	17.5	6.70		0.477	18.6	6.30		19.3	6.00		0.539	20.3	7.73	
Fat-free mass (kg)	23.4	3.84		0.246	24.6	4.90		28.2	5.58		0.119	30.5	6.98	
Fat-free mass index SDS	0.49	1.07	0.015	0.003	-0.19	0.89	0.159	0.15	1.03	0.439	0.121	-0.24	1.07	0.140
Fat-free mass hydration (%)	75.6	2.14		0.868	75.7	1.47		75.3	1.65		0.240	74.9	1.38	
Fat-free mass density (kg/L)	1.088	0.007		0.407	1.089	0.005		1.090	0.006		0.030	1.093	0.004	
Protein mass (kg)	4.33	0.68		0.345	4.55	1.13		5.27	1.01		0.071	5.83	1.48	
Protein mass index SDS	0.43	1.45	0.107	0.057	-0.12	1.06	0.427	0.18	1.12	0.375	0.555	0.03	1.04	0.838
Mineral mass (kg)	1.33	0.29		0.114	1.45	0.30		1.69	0.47		0.129	1.84	0.42	
Mineral mass index SDS	0.003	0.83	0.983	0.278	-0.22	0.87	0.108	-0.11	0.97	0.546	0.614	-0.23	1.04	0.156

P_{group} independent t -test; CF boys and control boys or CF girls and control girls; P_{ref} one-sample t -test compared to zero, CF and controls compared to reference data. SDS; standard deviation score, LS ; Lumbar spine (L2-L4) bone mineral apparent density which is a 3-D ‘volumetric’ measurement that is calculated from the 2-D Bone mineral density (BMD) actually measured by the DXA machine, SDS are calculated from reference data (204). Fat mass index (fat mass/height²), Fat-free mass index(fat-free mass/height²), Protein mass index (protein mass/height²), Mineral mass index (fat mass/height²). SDS are calculated from reference data collected in 533 contemporary healthy children (155). **Table continues on next page.**

Table 6.5 continued. Body composition of cystic fibrosis (CF) and control boys with group comparison (P_{group}) and compared to reference data (P_{ref}).

	Baseline							Two years						
	CF n=31				Control n=44			CF n= 31				Control n=44		
	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}
DXA soft tissue														
Whole body fat mass (kg)	5.01	2.99		0.437	5.58	3.20		7.06	3.64		0.223	8.37	5.04	
Whole body fat-free mass (kg)	23.4	3.62		0.167	24.8	4.79		28.0	5.50		0.145	30.16	6.86	
Trunk fat mass (kg)	2.03	1.38		0.811	2.10	1.36		3.04	1.81		0.290	3.82	3.83	
Trunk fat-free mass (kg)	11.2	1.71		0.972	11.3	2.09		13.4	2.48		0.549	13.0	2.90	
Limb fat mass (kg)	2.58	1.54		0.235	3.05	1.77		3.58	1.79		0.083	4.58	2.79	
Limb fat-free mass (kg)	9.01	1.82		0.021	10.3	2.56		11.4	2.81		0.016	13.3	3.67	

P_{group} ; independent t -test, CF boys and control boys or CF girls and control girls; **P_{ref}** ; one sample t -test compared to zero, CF and controls compared to reference data. SDS; standard deviation score, LS ; Lumbar spine (L2-L4) bone mineral apparent density which a 3-D ‘volumetric’ measurement that is calculated from the 2-D Bone Mineral Density (BMD) actually measured by the DXA machine, SDS are calculated from reference data (215). Fat mass index (fat mass/height²), Fat-free mass index(fat-free mass/height²), Protein mass index (protein mass/height²), Mineral mass index (fat mass/height²). SDS are calculated from reference data collected in 533 contemporary healthy children (155).

Table 6.6. Body composition of cystic fibrosis (CF) and control girls with group comparison (P_{group}) and compared to reference data (P_{ref}).

	Baseline							Two years						
	CF n=38				Control n=49			CF n= 38				Control n=49		
	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}
Body volume (L)	28.5	7.75		0.025	32.8	9.45		36.3	10.6		0.039	41.1	10.4	
Total body water (L)	17.3	4.10		0.066	19.1	4.90		21.1	5.02		0.026	23.7	5.46	
Total bone mineral content (kg)	1.03	0.26		0.014	1.21	0.37		1.35	0.37		0.014	1.59	0.50	
Total bone mineral density (g/cm^2)	0.88	0.08		0.272	0.90	0.08		0.94	0.07		0.244	0.90	0.17	
Total bone mineral density SDS	0.00	0.74	1.000	0.077	0.27	0.66	0.006	-0.20	0.84	0.148	0.265	0.03	1.03	0.847
LS bone mineral density (g/cm^2)	0.74	0.11		0.035	0.79	0.12		0.84	0.14		0.077	0.90	0.17	
LS bone mineral density SDS	-0.52	1.22	0.013	0.024	0.01	0.94	0.930	-0.66	1.19	0.005	0.005	0.04	1.02	0.804
LS bone mineral apparent density SDS	-0.44	1.28	0.042	0.119	-0.04	1.07	0.789	-0.40	1.07	0.026	0.135	-0.05	1.08	0.739
4-Component model														
Fat mass (kg)	6.53	3.08		0.010	8.60	4.03		9.28	4.87		0.106	10.9	4.52	
Fat mass index SDS	-0.63	0.82	0.001	0.026	-0.16	0.94	0.246	-0.55	1.13	0.010	0.126	-0.18	0.93	0.177
Fat (%)	21.2	5.25		0.014	24.4	6.53		23.4	7.31		0.301	25.0	6.71	
Fat-free mass (kg)	23.1	5.35		0.080	25.4	6.37		28.4	6.76		0.035	31.7	7.40	
Fat-free mass index SDS	-0.33	0.90	0.031	0.157	-0.05	0.91	0.715	-0.47	1.02	0.007	0.008	0.07	0.84	0.560
Fat-free mass hydration (%)	74.8	1.81		0.477	75.1	1.52		74.1	1.81		0.182	74.6	1.51	
Fat-free mass density (kg/L)	1.091	0.007		0.373	1.092	0.006		1.095	0.006		0.467	1.094	0.007	
Protein mass (kg)	4.53	1.16		0.315	4.77	1.12		5.63	1.42		0.185	6.05	1.45	
Protein mass index SDS	0.08	1.08	0.662	0.797	0.02	0.95	0.878	0.09	1.14	0.645	0.712	0.17	1.07	0.260
Mineral mass (kg)	1.31	0.32		0.009	1.55	0.47		1.74	0.48		0.019	2.03	0.64	
Mineral mass index SDS	-0.83	1.14	0.001	0.001	-0.01	0.98	0.965	-0.66	1.06	0.000	0.001	0.10	1.00	0.503

P_{ref} ; one-sample t -test CF and controls compared to zero, CF and controls compared to reference data (155), P_{group} ; independent sample t -test; CF boys and control boys and CF girls and control girls, SDS; standard deviation score, LS; Lumbar spine (L2-L4) bone mineral apparent density which is a 3-D 'volumetric' measurement that is calculated from the 2-D Bone Mineral Density (BMD) actually measured by the DXA machine, SDS are calculated from reference data (215). Fat mass index (fat mass/height²), fat-free mass index (fat-free mass/height²), protein mass index (protein mass/height²), mineral mass index (fat mass/height²). SDS are calculated from reference data collected in 533 contemporary healthy children (155).

Table continues on next page.

Table 6.6 continued. Body composition of cystic fibrosis (CF) and control girls with group comparison (P_{group}) and compared to reference data (P_{ref}).

	Baseline							Two years						
	CF n=31				Control n=44			CF n= 31				Control n=44		
	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}	Mean	SD	P_{ref}	P_{group}	Mean	SD	P_{ref}
DXA soft tissue														
Whole body fat mass (kg)	6.80	3.23		0.019	8.89	4.55		9.80	5.05		0.073	11.8	5.26	
Whole body fat-free mass (kg)	22.6	5.13		0.058	24.9	5.89		27.3	6.27		0.044	30.3	6.88	
Trunk fat mass (kg)	2.92	1.65		0.113	3.60	2.17		4.50	2.63		0.325	5.06	2.65	
Trunk fat-free mass (kg)	11.0	2.69		0.601	11.3	2.76		13.4	3.25		0.775	13.6	3.73	
Limb fat mass (kg)	3.44	1.56		0.003	4.78	2.37		4.81	2.38		0.011	6.22	2.60	
Limb fat-free mass (kg)	8.71	2.32		0.002	10.5	2.97		10.9	2.90		0.001	13.3	3.34	

P_{ref} ; one-sample t -test CF and controls compared to zero, CF and controls compared to reference data (155), P_{group} ; independent sample t -test; CF boys and Control boys or CF girls and Control girls SDS; standard deviation score, LS ; Lumbar spine (L2-L4) bone mineral apparent density which is a 3-D ‘volumetric’ measurement that is calculated from the 2-D Bone Mineral Density (BMD) actually measured by the DXA machine, SDS are calculated from reference data (215). Fat mass index (fat mass/height²), fat-free mass index(fat-free mass/height²), protein mass index (protein mass/height²), mineral mass index (fat mass/height²). SDS are calculated from reference data collected in 533 contemporary healthy children.(155)

6.6.3.2 Compared to control group

Boys with CF did not differ significantly from the control boys at baseline apart from having a higher FFMI SDS ($p<0.05$), and at the 2 year measurement they had higher total BMD ($p<0.01$) and FFM density ($p<0.05$). Comparison in the girls revealed more differences than the boys with CF girls lower in total BMC, LS BMD SDS ($p<0.05$) and lower MMI SDS ($p<0.001$) at both time-points. Size adjusting LS BMD made the difference non-significant although the CF girls had a lower mean value. The CF girls had lower FM although this was only significant at baseline ($p<0.05$) and lower FFM although this was only significant at 2 years ($p<0.01$).

DXA regional soft tissue analysis revealed that the boys with CF had significantly less limb FFM at both time points $p<0.05$ despite whole body and trunk FFM not being significantly different from controls. Girls with CF had less whole body FM and FFM which appears to be due to deficits in the limbs at both time-points.

6.6.3.3 Potential confounders affecting body composition outcomes

Table 6.7 shows adjusted mean differences in body composition (CF- control) after adjustment for age, puberty and height for non-indexed variables at baseline and 2 years with a summary in **Table 6.8**. CF boys had greater weight, BMI, FFMI SDS and waist circumference at both time points although this was non-significant for BMI SDS at 2 years ($p<0.05$). They were also shorter by on average, 0.3 SDS although this was non-significant and had lower density of FFM at 2 years ($p<0.05$). Girls with CF were significantly shorter with lower BMI SDS, FMI SDS and MMI SDS at both time points ($p<0.05$). FFMI SDS and MUAC were lower in CF girls at baseline (non-significant) and became increasingly lower at 2 years ($p<0.05$). To determine whether differences in body composition were due to different levels of activity the parent's rating of the child's activity level was added to the model with no effect on the outcome (data not shown).

DXA regional body composition indicated that boys with CF had significantly more whole body FFM at baseline ($p<0.05$) and more at 2 years (non-significant). There was a much larger difference in the trunk, boys with CF had greater FFM at baseline and 2 years ($p<0.001$). Despite whole body FFM being similar in the girls, the girls with CF appear to have much more FFM in the trunk than control girls ($p<0.005$) at both time-points and less in the limbs ($p<0.001$). The non-significant deficit of whole body FM is attributable to deficits of FM in the limbs ($p<0.05$).

Table 6.7. Difference in size and whole-body composition (cystic fibrosis (CF) minus control) at baseline and two years. Positive mean values indicate greater values in children with CF.

	Boys (CF = 31, control = 44)						Girls (CF = 38, control = 49)					
	Baseline			Year 2			Baseline			Year 2		
	Mean	SEM	P	Mean	SEM	P	Mean	SEM	P	Mean	SEM	P
Weight (kg)	1.87	0.74	0.013	1.95	1.10	0.079	-0.77	0.92	0.407	-1.55	1.30	0.238
Weight SDS	0.36	0.14	0.013	0.33	0.15	0.036	-0.17	0.15	0.273	-0.31	0.18	0.082
Height (m) ¹	-1.79	1.26	0.159	-2.34	1.68	0.170	-5.04	1.23	<0.001	-6.22	1.33	<0.001
Height SDS ¹	-0.32	0.21	0.131	-0.31	0.23	0.183	-0.78	0.20	<0.001	-0.97	0.20	<0.001
BMI (kg/m ²) ¹	0.87	0.39	0.030	0.76	0.50	0.134	-0.96	0.43	0.030	-1.38	0.53	0.010
BMI SDS ¹	0.49	0.21	0.020	0.43	0.24	0.072	-0.42	0.21	0.043	-0.63	0.22	0.005
Total body water (L)	1.04	0.36	0.005	1.14	0.57	0.050	-0.03	0.37	0.943	-0.68	0.47	0.150
Body volume (L)	1.85	0.76	0.018	1.35	1.31	0.307	-0.83	0.97	0.391	-1.49	1.35	0.275
4C Fat mass (kg)	0.55	0.61	0.370	0.64	0.89	0.475	-0.88	0.66	0.185	-0.72	0.94	0.447
4CFat mass index (kg/m ²) ¹	0.17	0.32	0.604	0.17	0.40	0.683	-0.75	0.31	0.016	-0.67	0.38	0.078
4C Fat mass index SDS ¹	0.08	0.24	0.753	0.16	0.22	0.486	-0.41	0.19	0.033	-0.42	0.21	0.050
4C Fat-free mass (kg)	1.28	0.50	0.013	1.31	0.75	0.085	0.08	0.46	0.865	-0.83	0.65	0.203
4C Fat-free mass index SDS ¹	0.71	0.24	0.004	0.61	0.25	0.019	-0.21	0.19	0.282	-0.58	0.20	0.005
4C Fat-free mass hydration (%)	0.11	0.44	0.798	0.46	0.38	0.231	-0.33	0.41	0.426	-0.21	0.40	0.594
4C Fat-free mass density (kg/L)	-0.001	0.001	0.347	-0.003	0.001	0.029	0.000	0.001	0.721	0.001	0.001	0.360
4C Protein mass (kg)	0.19	0.18	0.285	0.11	0.20	0.574	0.19	0.13	0.133	-0.07	0.20	0.740
4C Protein mass index SDS ¹	0.50	0.31	0.107	0.28	0.27	0.291	0.07	0.22	0.741	-0.11	0.24	0.657
4C Mineral mass (kg)	0.05	0.03	0.121	0.07	0.06	0.207	-0.09	0.04	0.029	-0.09	0.06	0.139
4C Mineral mass adjusted (kg) ²	0.03	0.03	0.402	0.05	0.06	0.394	-0.10	0.04	0.015	-0.10	0.05	0.057
4C Mineral mass index SDS ¹	0.29	0.21	0.171	0.26	0.25	0.304	-0.74	0.23	0.002	-0.77	0.22	0.001

General linear model, adjusting for group and puberty as fixed factors and age and height as continuous variables. **P**; independent sample *t*-test, CF v control, SDS; standard deviation score, 4C; 4-component model, ¹adjusted for age and puberty only, ²adjusted for lumbar spine bone area. **Table continues on next page.**

6.7 continued. Difference in size and whole-body composition (CF minus control) at baseline and two years. Positive mean values indicate greater values in children with CF.

	Boys (CF = 31, control = 44)						Girls (CF = 38, control = 49)					
	Baseline			Year 2			Baseline			Year 2		
	Mean	SEM	P	Mean	SEM	P	Mean	SEM	P	Mean	SEM	P
DXA LS bone mineral density	0.01	0.02	0.456	0.01	0.02	0.540	-0.01	0.02	0.609	-0.01	0.02	0.596
DXA LS bone mineral density SDS	0.13	0.18	0.476	0.15	0.21	0.464	-0.01	0.21	0.952	-0.14	0.24	0.554
DXA LS bone mineral apparent density SDS	-0.01	0.21	0.947	0.07	0.25	0.794	-0.19	0.27	0.497	-0.16	0.26	0.547
DXA whole body fat mass (kg)	0.72	0.60	0.231	0.72	0.97	0.458	-0.82	0.70	0.246	-1.07	1.00	0.287
DXA whole body fat-free mass (kg)	1.08	0.47	0.026	1.23	0.84	0.151	-0.06	0.40	0.885	-0.35	0.55	0.529
DXA trunk fat mass (kg)	0.48	0.27	0.082	0.19	0.72	0.796	-0.06	0.35	0.866	-0.07	0.52	0.893
DXA trunk fat-free mass (kg)	1.04	0.24	<0.001	1.78	0.51	0.001	0.80	0.22	<0.001	1.16	0.40	0.005
DXA limb fat mass (kg)	0.23	0.32	0.473	0.11	0.51	0.825	-0.73	0.36	0.046	-0.98	0.49	0.048
DXA limb fat-free mass (kg)	-0.003	0.25	0.992	-0.10	0.41	0.806	-0.77	0.22	0.001	-1.22	0.31	<0.001
Waist circumference (cm)	5.20	0.94	<0.001	4.96	1.16	<0.001	1.26	1.11	0.261	1.47	1.36	0.285
Waist circumference SDS	0.93	0.15	<0.001	0.69	0.18	<0.001	0.25	0.19	0.185	0.21	0.20	0.311
Hip circumference (cm)	0.80	0.92	0.389	0.60	1.18	0.613	-2.06	1.06	0.056	-2.39	1.25	0.060
Mid-upper arm circumference (cm)	0.03	0.54	0.959	0.11	0.56	0.846	-0.88	0.56	0.118	-1.31	0.61	0.036
Log 4 skinfold thickness ³	0.09	0.08	0.250	0.03	0.10	0.774	-0.06	0.09	0.496	-0.10	0.10	0.302
4 skinfold thickness SDS ⁴	0.19	0.19	0.327	0.12	0.20	0.543	-0.12	0.21	0.558	-0.25	0.23	0.265

General linear model, adjusting for group and puberty as fixed factors and age and height as continuous variables. **P**; independent *t*-test, CF v control, SDS; standard deviation score, LS; lumbar spine bone mineral apparent density (size adjusted bone mineral density) SDS. ³Log 4 skinfolds (bicep, tricep, subscapular and supra-iliac). ⁴Mean of bicep, tricep, subscapular and supra-iliac SDS, ^{3,4} n= CF boys 31, control boys 42, CF girls 37, control girls 46 at baseline and 2 years n= CF boys 31, control boys 43, CF girls 37, control girls 48.

Table 6.8. Summary of comparisons (before and after adjustment for age, puberty and height¹)

Mode of analysis	Boys with CF compared to;						Girls with CF compared to;					
	Unadjusted Group-match		Unadjusted Reference		Group-match adjusted for, age, puberty and height ¹		Unadjusted Group-match		Unadjusted Reference		Group-match adjusted for age, puberty and height ¹	
Year	0	2	0	2	0	2	0	2	0	2	0	2
Weight SDS ¹	NS	NS	NS	NS	↑	↑	↓	↓	↓	↓	NS	NS
Height SDS	↓	↓	↓	↓	NS	NS	↓	↓	↓	↓	↓	↓
BMI SDS	NS	NS	NS	NS	↑	NS	↓	↓	NS	NS	↓	↓
Waist SDS ¹	↑	↑	↑	↑	↑	↑	NS	NS	↑	↑	NS	NS
Fat mass index SDS	NS	NS	NS	NS	NS	NS	↓	NS	↓	↓	↓	↓
Fat-free mass index SDS	↑	NS	↑	NS	↑	↑	NS	↓	↓	↓	NS	↓
Protein mass index SDS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mineral mass index SDS	NS	NS	NS	NS	NS	NS	↓	↓	↓	↓	↓	↓

Unadjusted comparisons using independent t-test for group-match and one sample t-test for reference data. Adjusted comparisons are a general linear model adjusted for age, puberty (pre-pubertal; Tanner stage1 versus Tanner stages 2-5) and ¹ height for weight and waist SDS. Reference data is UK 1990 (54;161;209) for weight, height, BMI and waist and reference data from 533 children measured by the 4-component model (155). Fat mass index (fat mass/height²), fat-free mass index (fat-free mass/height²), protein mass index (protein mass/height²), mineral mass index (mineral mass/height²). Highlights signify disagreement.

6.6.4 Relationship between body composition and spirometry

Mean FEV₁ SDS was; CF boys, mean (SD), baseline -1.10 (1.54) and 2 years -1.15 (1.56) (Table 2) and CF girls, baseline -1.56 (1.44) and 2 years -2.05 (1.58) (Table 6.3). There was a significant deterioration in the girls lung function ($p < 0.01$) between baseline and 2 years of 0.5 SDS.

Separate simple regression analysis of FEV₁ SDS and BMI SDS or FM or FFM or MM or whole body BMC or BMD were performed with height in the model (except for BMI) and additionally adjusting MM, and BMC for LS bone area (**Table 6.9**) at baseline and 2 years. There were no significant associations in boys apart from FFM ($p < 0.05$) at the 2 year measurement. Only FM at baseline and total BMC at 2 years were associated with lung function in girls ($p < 0.05$). Whole body BMD unadjusted for height was also included so that this study is comparable with studies that have not adjusted for height.

Table 6.9. Simple regression analysis of factors (adjusted for height)¹ associated with forced expired volume in 1 sec standard deviation score

	Boys n=31					Girls n=38				
	<i>B</i>	SEM	<i>t</i>	<i>P</i>	<i>r</i> ²	<i>B</i>	SEM	<i>t</i>	<i>P</i>	<i>r</i> ²
Year 0										
BMI SDS	0.30	0.32	0.94	0.355	0.004	0.43	0.25	1.76	0.087	0.055
Fat mass (kg)	0.01	0.13	0.10	0.919	0.067	0.23	0.11	2.21	0.034	0.075
Fat-free mass (kg)	0.02	0.17	0.11	0.912	0.067	-0.03	0.15	-0.23	0.820	0.055
Fat-free mass (kg)	-0.04	0.17	-0.23	0.818	0.022	-0.02	0.16	-0.11	0.911	0.084
Mineral mass (kg) ²	1.79	2.42	0.74	0.465	0.040	1.97	2.08	0.94	0.352	0.056
DXA bone mineral content (kg) ²	2.29	3.06	0.75	0.460	0.040	4.53	2.71	1.67	0.104	0.001
DXA bone mineral density (g/m ³)	0.20	5.98	0.03	0.974	0.067	0.33	3.55	0.09	0.927	0.057
Year 2										
BMI SDS	0.47	0.30	1.58	0.126	0.049	0.36	0.23	1.54	0.133	0.037
Fat mass (kg)	0.003	0.11	0.03	0.977	0.061	0.09	0.08	1.17	0.249	0.014
Fat-free mass (kg)	0.31	0.12	2.55	0.017	0.145	0.03	0.12	0.28	0.781	0.052
Fat-free mass (kg)	0.21	0.13	1.60	0.121	0.190	0.06	0.13	0.48	0.634	0.072
Mineral mass (kg) ²	2.51	1.55	1.63	0.115	0.192	3.15	1.70	1.86	0.073	0.022
DXA bone mineral content (kg) ²	3.12	2.00	1.56	0.131	0.183	4.87	2.01	2.42	0.021	0.084
DXA bone mineral density (g/m ³)	7.09	5.14	1.38	0.179	0.009	6.73	6.03	1.12	0.272	0.018

SDS; standard deviation score, DXA; dual-energy X-ray absorptiometry, ¹except BMI SDS, ² additionally adjusted for lumbar spine bone area

6.6.5 Body composition assessed by simple techniques

Body composition assessed by SFT, BIA, DXA and TBW and a comparison with the 4CM is presented in Chapter 8.

6.7 Discussion

The findings of this analysis confirm the clear sex differences presented in Chapter 5 where deficits in FM and FFM including MM are apparent in the girls with CF whereas the boys with CF have body composition much closer to that of controls.

The aims of this chapter were to test the hypotheses that; (1) there is no significant difference in body composition at baseline but differences will be apparent over time, (2) that specific components of body composition are associated with lung function in children with CF and (3) that the conclusions are the same regardless of the mode of analysis. This chapter relates to chapter 5 because it investigates the body composition and lung function of children with CF at baseline, albeit a smaller number of CF children compared to a larger number of control children. The reduction in numbers of CF children is due to the fact that only children who have 2 complete 4CM measurements at baseline and 2 years are included. The reduced number of boys in this baseline analysis as opposed to the baseline analysis in Chapter 5 means that the average FFM has increased and average FM decreased in boys although this does not affect the outcome of the group comparison. The group mean for body composition variables in the CF girls are similar.

Analyses in this chapter are cross-sectional at baseline and 2 years using a control group and using a large reference population. The control group comparison, although smaller in number than the reference group has the advantage of being contemporary although may not be considered 'normal', for example, control girls in this study have on average 0.4 SDS greater weight and height than the 1990 UK

reference data. The group matching also allows for comparison of change over time (Chapter 7). Comparison to a reference population assures that the children with CF are compared to standardised controls but has the disadvantage that reference data becomes out-of-date. The 1990 UK reference data used in this study for anthropometric comparison was collected in 1988 and the body composition and SFT data was collected from 2001-2007. However, in a clinical situation comparison with a reference population is the most practical method.

Although one study reported that children with CF do not have delayed puberty (36) others report delayed puberty (35;220). In this study the boys with CF have delayed puberty compared to the healthy boys but surprisingly, although there are a few more pre-pubertal CF girls at baseline, (68% compared to 61%) at aged 8-14 years there are slightly more controls (29% control compared to 27% CF) that are pre-pubertal. It is difficult to find an explanation since the CF girls are smaller with lower FMI and FFMI SDS than control girls. If the CF and control girls are divided by Tanner stage 1 and 2 compared to Tanner stage 3 and above then the results are; stage 1 and 2, CF 66%, control 59%, Tanner stage ≥ 3 , CF 34%, control 41%. This indicated that there are more control girls in the later stages of puberty than girls with CF and this is a stage when the effect of growth and development are the greatest. It is also possible that the girls with CF may be overestimating their own development in line with that of their peer group. An alternative explanation from an evolutionary biology perspective would suggest that the less likely an organism is to live then the earlier menarche occurs in order to produce offspring earlier (221).

6.7.1 Regarding hypothesis 1; that there will be no significant differences in body composition at baseline but differences will become apparent over time.

The findings that at baseline the girls with CF have altered body composition does not support hypothesis 1 although the boys with CF appear to be similar to control children. After 2 years boys with CF had a reduction in FFM and girls remain with deficits of FM, FFM and MM.

In accord with previous research (64;72;100) both boys and girls with CF are short at both time points. The smaller hip and mid-upper arm circumference of the children with CF suggests they are less mature than their healthy counterparts.

All the children in this study had waist circumferences greater than the 1990 UK reference data, however, the group mean was greater in the CF children compared to controls, possibly reflecting inflammation and/or enlargement of the abdominal organs. In the absence of higher FM by 4CM or raised SFT it is unlikely that this large waist circumference is due to fat. Regional FM and FFM by DXA indicated that boys with CF had more overall FFM and that the difference was totally in the trunk at both time-points. Despite the girls with CF having similar FFM to controls they also appeared to have much more FFM in the trunk and an equal deficit in the limbs. Compared to controls the girls with CF also have a large deficit of FM in the limbs (-0.7 kg baseline and -1.0 kg at 2 years). DXA regional soft tissue SDS would aid the understanding of regional differences.

The reason for identifying high FFM in the trunk may be a true increase or due to methodological problems. It is possible that increased FFM in the trunk is related to bowel dysfunction; abdominal pain and discomfort are common in CF with long gut transit times reported (222), sub-mucosal fibrosis and stasis of faecal material (223) and thickened mucosal covering of the bowel (224) which are all possible reasons for the enlarged bowel and waist circumference identified in this group. In addition other organs may be enlarged; right ventricle enlargement as a result of pulmonary hypertension and liver and spleen enlargement due to hepatobiliary obstruction (225). One might suppose that since the children in this study are young the number and severity of these symptoms will be limited; only 1 child had liver disease diagnosed at baseline and 7 by the second measurement. However, it is also likely that these problems are preceded by asymptomatic changes and these, combined with inflammation and oedema may account for the distended abdomen.

Methodological problems may have some impact on these findings. DXA measurements have been shown to be biased in extremes of fatness and thinness

(102;196) which may have several causes. First, an assumed constant for hydration (226); second, subject size may influence bias through the effect of tissue depth (196) with increasing tissue depth associated with increasing bias by DXA (227); third, soft tissue distribution may affect accuracy because pixels containing bone extrapolate soft tissue composition from adjacent regions which may have a different composition of FM and FFM from the region overlying the bone (196). In the trunk region there are many more bone to non-bone pixels than other parts of the body and this, coupled with a distended abdomen and possible oedema may, in part explain the findings of large increases in FFM in the trunk of children with CF. Comparative studies between DXA and MRI may illuminate the question. However, partial correlations suggest that the variability in the waist circumference of the children with CF cannot be explained by FM and FFM to the same extent as in controls, (R^2 ; CF FM 67%, FFM 28% and controls FM 81%, FFM 36%). Body composition is not exerting the same influence on waist circumference in CF children as in controls.

Once an adjustment for age, height and puberty has been made the boys with CF differ from the controls at baseline having more FFM. At 2 years there is no longer a significant difference in FFM except where the comparison is made using FFM indexed and converted to SDS. This is an indication of the differing outcome depending on whether raw body composition variables (adjusted for actual height), indexed variables (adjusted for height²) or SDS are used in the analysis. These findings are contrary to expectation, the boys with CF have more FFM at baseline and at 2 years although no longer statistically significant. In this study the girls with CF have a deficit of FM at baseline which appears stable over time. The girls with CF group-mean FFMI SDS deteriorates over time although the mineral component is stable and low suggesting deterioration in the non-mineral lean mass.

6.7.2 Regarding hypothesis 3, that the conclusions are the same regardless of the mode of analysis.

The question of whether it is better to compare the CF children directly to a group of controls or whether to compare to a larger reference population using SDS is debatable. Comparison with a smaller contemporary control group has the advantage of comparing two groups of children measured at a similar time and allows for comparison of change in one group compared to change in the other over time. Comparison with a reference population is preferable in a clinical setting.

Comparison between pair and group-match is difficult in this study because there is a reduced number in the group-match. The boys at baseline in the pair-match (Chapter 5) are heavier and taller than the reduced number of boys in the group-match analysis (Chapter 6). However, at both analyses they are short (pair-match, -0.41 height SDS, group-match -0.51). The girls with CF have similar mean BMI SDS in the pair (-0.33) and group (-0.26) analyses. The question of whether it is better to compare a patient group (pair or group-match) to a control group or to a reference group is inevitably affected by how typical the control group is. Clearly, the larger the number of control children, the more likely their mean value is typical of healthy children and therefore, the use of large reference populations, particularly when they are in a similar ethnic group, era or geographic area has even greater benefits. Such reference populations allow for the calculation of SDS thereby allowing comparison of the child to a standard and allowing assessment of change over time. In addition, comparison can still be made by pair or group analysis by first standardizing both patient and controls to the large reference population by calculating SDS. Table 6.8 based on comparison of SDS against reference and group-match and without and with adjustment for age, height and puberty indicated that in boys, different outcomes are obtained dependent on whether adjustment for age, height and puberty has been made. In the analyses of girls' data the picture is less clear which may, in part be due to the control girls being taller and heavier than average.

6.7.3 Regarding hypothesis 2, that specific components of body composition are associated with spirometry in children with cystic fibrosis

Girls had poorer lung function than boys, which deteriorated over two years. This may be a reflection of their sub-optimal body composition and prognosis. In accord with many previous studies FFM was significantly associated with lung function in boys only at 2 years. There was no such relationship in girls with both FM at baseline and total BMC at 2 years positively associated with FEV₁ possibly a reflection of, or a contribution to their poor nutritional status.

6.8 Study limitations

As acknowledged in the previous chapter I used predicted rather than measured lung volume when calculating body volume by ADP. However, a study of children with CF deriving FM from ADP concluded that there was no significant difference in FM calculations between measured or predicted lung volumes (219).

Since exercise will affect the amount of lean tissue we repeated the analyses taking into account parental reported exercise level; there was no difference in outcome. However, the parental rating may be affected by differing expectations in healthy and chronically ill patients and between the sexes. In future studies it will be informative to use an objective measure of physical activity.

This study compares the same children at 6-12 and 8-14 years and therefore the numbers are reduced from the original analysis of 85 to 69. However, this would be sufficient to detect a 0.48 SDS difference between groups which is likely to be clinically important.

6.9 Summary

- There were clear sex differences at baseline, with CF boys having more FFM and girls with CF have less FM and MM than the reference population. This is consistent with the findings in the larger cohort presented in Chapter 5.
- After 2 years FFMI SDS is lower in boys (although above zero) and the girls remain stable for FM and MM but have lower non-osseous FFM.
- Increased waist circumference in children with CF is accompanied by increased FFM in the trunk as assessed by DXA in the absence of increased FM by 4CM or SFT. The nature of abdominal distension may be better identified using MRI.
- The findings of this study are that, unlike previous research it is FM that relates to lung function at baseline in the girls only. Consistent with previous studies I found a relationship between FFM and lung function in boys at age 8-14 years. Total BMC also related to lung function in 8-14 year old girls with CF.
- The sub-optimal body composition in girls may be a reflection of their poor lung function.
- My findings do not support the hypothesis that the conclusions are the same regardless of whether matched-pairs, group comparisons or comparison with a reference population are used for comparison. As a consequence it is difficult to compare the findings of studies using different methods of analysis and for clinical practice the solution is likely to be a pragmatic one. Clinically, the simplest method to identify individual children with sub-optimal body composition and to monitor them over time is to use SDS.
- The limitations of each method of analysis need to be acknowledged and the fact that statistical significance does not necessarily equate to clinical significance needs to be considered.

Chapter 7. Longitudinal comparison of two-year change in body composition of children with cystic fibrosis with that of healthy children and the relationship between change in body composition and lung function in children with cystic fibrosis.

7.1 Introduction

Although there are several reports of longitudinal growth (height and weight) in children with CF (10;13;50;122;141) there are few studies that address the changes in body composition over time (36;65;67;96) and none that use a ‘criterion’ body composition technique. This chapter outlines the change in body composition over 2 years in CF children age 6-12 years using the 4CM and compares the change to that of healthy children. The relationship between change in body composition and change in lung function in CF children is also presented. The subjects and methods are the same as those presented in the cross-sectional analysis (Chapter 6) and **Figure 7.1** shows the relationship between the analyses and the subjects in Chapters 5, 6 and 7.

<u>Chapter 5</u>	<u>Chapter 6</u>	<u>Chapter 7</u>
Cross-sectional, comparison with reference data and pair-match analyses 85 CF, 85 Control	Cross-sectional, comparison with reference data and group-matched analyses 69 of 85CF, 72 of 85Control + 21	Longitudinal comparison with reference data and group-match analyses. 69 of 85 CF 72 of 85 Control + 21

Figure 7.1. Description of mode of analyses and subjects in Chapters 5-7.

The hypothesis addressed in this chapter is that; baseline and change in specific components of body composition are associated with disease severity and predict clinical outcome.

7.2 Study design

This is a longitudinal study comparing the 2 year change in body composition of both children with CF and age matched (6-12 years at baseline) controls. Change was investigated in both absolute and conditional (that is unrelated to the baseline) terms. In addition, baseline and conditional change in lung function, measured on the same day (or within 4 weeks if not possible) was related to conditional change in body composition in the children with CF.

7.3 Recruitment and exclusion criteria

Children with CF were recruited from patients at GOSH and the control children from an on-going study of healthy children at UCL, ICH. Details can be found in Chapter 4.

7.4 Methods

Measures of anthropometry and BMC, TBW and densitometry for 4CM were performed as described previously. LS and whole body BMC and BMD were measured by DXA to assess bone status. Confounding factors such as pubertal status and activity were assessed with questionnaires, the details of all these can be found in Chapter 4 and Appendices 5 and 6. The children with CF also had lung function assessed, described in Chapter 4.

7.5 Statistical analyses

7.5.1 Size adjustment and standard deviation score calculation

Characteristics of all the children were compared to 1990 UK reference data to generate SDS for weight, height, BMI and waist circumference. Size adjustment of body composition variables was made as described in 4.6.2. and SDS were calculated compared to a contemporary reference population for FM, FFM, PM, MM and SFT. Total and LS BMD SDS were generated from the Lunar Prodigy software using machine reference data matched for age, sex and ethnic group and a size adjusted BMAD SDS was calculated as described in 4.6.2. Conditional change in anthropometric and body composition variables were calculated by generating a prediction equation for each parameter using data from the controls and then calculating the residuals (predicted – actual) for children with CF as described in 4.6.6. Conditional change in FEV₁ was calculated using regression analysis in the children with CF only.

7.5.2 Comparison of change in anthropometric and body composition variables

Initial analysis indicated a strongly significant difference between the sexes and therefore data for males and females were analysed separately.

Change between baseline and two years in CF and controls separately was investigated; (i) using paired *t*-tests between baseline and 2 year variables in both CF and control children (absolute change) and (ii) using one-sample *t*-tests for the difference between conditional change (change other than that expected if the CF child is tracking growth and body composition of the control group) and zero in CF children only. Group means for absolute and conditional change would be similar in the control children because the regression equation was derived from this group.

(iii) Difference in change (2 year – baseline) between CF and control children was investigated using independent *t*-tests.

Summary of comparison of change analysis

- | | |
|---|---------------------------|
| 1) CF absolute change 0-2 years | <i>paired t-test</i> |
| 2) Control absolute change 0-2 years | <i>paired t-test</i> |
| 3) CF conditional change 0-2 years | <i>1 sample t-test</i> |
| 4) CF absolute change compared to control absolute change | <i>independent t-test</i> |

7.5.3 Effect of predictors of change on anthropometric and body composition variables

To identify possible predictors of conditional change in body composition variables for inclusion in the regression models, Pearson's correlation and Spearman's rho, (for skewed data) was performed for data of children with CF. Factors included were; spirometry, activity, anthropometric and body composition variables at baseline and conditional change. In addition pubertal status at baseline and change in pubertal status and genotype were included. Multiple backwards regression analysis was used separately to investigate predictors of conditional change in FMI or FFMI or MMI SDS.

A general linear model was used to investigate any significant effect of CF on change in height, weight, BMI, FMI, FFMI or MMI SDS with the 2 year measurement as the dependent variable and the baseline measurement as the independent variable with condition (CF=1, control =0) as a fixed factor. Considering the significant associations a separate general linear model was performed to investigate any interactions between CF and baseline measurements.

Summary of effect of predictors analyses

- 1) Correlation analysis in CF children to identify possible predictors of conditional change in body composition variables for inclusion in subsequent multi-variant analyses.
- 2) Multiple backwards regression analysis to identify predictors of conditional change in FMI SDS, FFMI SDS and MMI SDS in CF and control separately.
- 3) General linear model to investigate the possible effect of having CF on absolute change in anthropometric and body composition variables.
- 4) Considering the results of 3), general linear model to test for any interaction between having CF and the baseline measurement in relation to the final measurement.

7.5.4 Spirometry

Correlation (Pearson's for normally distributed and Spearman's rho for skewed data) analysis was used to identify significant associations between absolute change in FEV₁ SDS and baseline and conditional change in activity, anthropometric and body composition variables, puberty and genotype. Significant factors were included in regression analyses to investigate the relationship between body composition variables and conditional change in FEV₁ SDS in the children with CF. Conditional change in FEV₁ SDS was calculated from the CF children only because the control children did not perform spirometry.

7.6 Results

7.6.1 Subjects

The same 69 (38 girls) CF and 93 (49 girls) control children, who all had 2 complete 4CM measurements and were included in the cross-sectional analysis of Chapter 6 were included in this analysis.

7.6.2 Change in anthropometry and body composition

Group absolute and conditional change in anthropometric and body composition variables is shown in **Table 7.1** (boys) and **Table 7.2** (girls). Change in FMI SDS (**Figure 7.2**) in children with CF was variable and a graph showing change in FFMI SDS (**Figure 7.3**) indicates that many of the children have declining FFMI SDS.

7.6.2.1 Absolute change in body composition

For SDS, which are comparable over time, control boys showed upward centile crossing in weight SDS ($p<0.05$) and SFT SDS ($p=0.001$) and downward centile crossing for LS BMD SDS ($p<0.05$) but not size adjusted BMAD. Boys with CF had, on average, downward centile crossing for waist SDS ($p<0.05$) and FFMI SDS ($p<0.01$). Both control and CF girls showed upward centile crossing waist ($p<0.01$) and SFT SDS ($p<0.05$). **Table 7.3** shows the mean difference in absolute change (2 year – baseline) between CF and control children (CF – control). The significant differences were, boys; age difference (mean (SD), CF; 2.08(0.13), control; 2.03(0.06)), waist SDS, (CF; -0.20 (0.46), control; 0.05 (0.45)), body volume, (CF; 6.25(3.07), control; 8.53(5.56)) and FFMI SDS, (CF;-0.35 (0.67), control; -0.05 (0.51)) and girls FFMI SDS, (CF; -0.14 (0.53), control; 0.11 (0.46)).

7.6.2.2 Conditional change in body composition

Conditional change in control children is not presented in the tables 7.1 and 7.2 because, as expected, the group mean is approximately zero. Conditional change in

boys with CF indicated significantly lower than expected gain in BMI SDS, waist SDS ($p<0.01$) and FFMI SDS ($p<0.05$), that is to say that they were not following the control group pattern of growth and lean tissue acquisition. However, SFT SDS gains were higher than expected ($p=0.051$). Girls with CF appear to be tracking the control girls' growth and body composition (albeit with lower values) apart from values for FFMI SDS which appear to be reducing ($p<0.001$). They also have a lower change in exercise time, an average of 1 hour 20 minutes less than the control girls ($p<0.05$).

Table 7.1. Change between baseline and two years in boys

	CF n=31						Control n= 44		
	Absolute change			Conditional change			Absolute change		
	Mean	SD	P	Mean	SD	P	Mean	SD	P
Age (y)	2.08	0.13	<0.001				2.03	0.06	<0.00
Weight SDS	-0.04	0.49	0.682	-0.18	0.50	0.060	0.14	0.38	0.018
Height SDS	0.10	0.29	0.073	-0.01	0.29	0.784	0.09	0.34	0.079
BMI SDS	-0.14	0.54	0.153	-0.28	0.57	0.010	0.10	0.53	0.211
Waist SDS	-0.20	0.46	0.024	-0.26	0.46	0.004	0.05	0.45	0.443
4 skinfold thickness SDS ¹	0.06	0.61	0.565	0.21	0.58	0.051	0.29	0.53	0.001
LS bone mineral density SDS	-0.10	0.47	0.197	0.02	0.43	0.806	-0.12	0.34	0.018
LS bone mineral apparent density SDS	0.10	0.43	0.254	0.16	0.46	0.072	-0.07	0.66	0.491
Fat mass index SDS	0.05	0.69	0.702	-0.07	0.60	0.534	0.11	0.64	0.279
Fat-free mass index SDS	-0.35	0.67	0.007	-0.24	0.66	0.047	-0.05	0.51	0.517
Protein mass index SDS	-0.25	1.15	0.236	-0.16	0.88	0.320	0.16	0.93	0.260
Mineral mass index SDS	-0.11	0.44	0.171	-0.01	0.44	0.893	-0.01	0.49	0.893
FEV ₁ SDS	-0.06	0.95	0.749						
Activity (hours)	-0.19	7.58	0.888	-0.27	5.33	0.783	0.80	5.26	0.322

SDS; standard deviation score, ¹ n=30 for CF, LS; lumbar spine, bone mineral apparent density; size adjusted (bone area) bone mineral density, FEV₁, forced expiratory volume in 1 sec. *P*; paired *t*- test for absolute change and 1 sample *t*-test against zero for conditional change.

Table 7.2. Change between baseline and two years in girls

	CF n= 38						Control n=49		
	Absolute change			Conditional change			Absolute change		
	Mean	SD	P	Mean	SD	P	Mean	SD	P
Age (y)	2.06	0.22	<0.001				2.04	0.14	<0.00
Weight SDS	-0.01	0.43	0.866	-0.11	0.46	0.170	0.03	0.28	0.420
Height SDS	0.06	0.45	0.422	-0.15	0.47	0.060	0.10	0.39	0.077
BMI SDS	-0.05	0.53	0.559	-0.10	0.54	0.261	0.01	0.35	0.799
Waist SDS	0.24	0.51	0.007	0.10	0.57	0.505	0.19	0.46	0.007
4 skinfold thickness SDS ¹	0.23	0.63	0.036	0.05	0.60	0.595	0.15	0.47	0.036
LS bone mineral density SDS	-0.14	0.76	0.279	-0.17	0.74	0.176	0.025	0.59	0.768
LS bone mineral apparent density SDS	0.03	0.72	0.773	0.02	0.66	0.857	-0.01	0.54	0.890
Fat mass index SDS	0.08	0.70	0.473	0.05	0.72	0.688	-0.03	0.45	0.690
Fat-free mass index SDS	-0.14	0.53	0.104	-0.30	0.54	0.001	0.12	0.45	0.075
Protein mass index SDS	0.01	1.21	0.965	-0.13	1.09	0.466	0.15	0.85	0.216
Mineral mass index SDS	0.16	0.74	0.185	-0.04	0.69	0.715	0.10	0.50	0.154
FEV ₁ SDS	-0.49	0.92	0.003						
Activity (hours)	-1.37	4.56	0.073	-1.28	3.23	0.019	-0.06	3.96	0.914

SDS; standard deviation score, ¹ n=36 for CF and 48 for control, LS; lumbar spine, bone mineral apparent density; size adjusted (bone area) bone mineral density, FEV₁, forced expiratory volume in 1 sec. *P*; paired *t*- test for absolute change and 1 sample *t*- test against zero for conditional change.

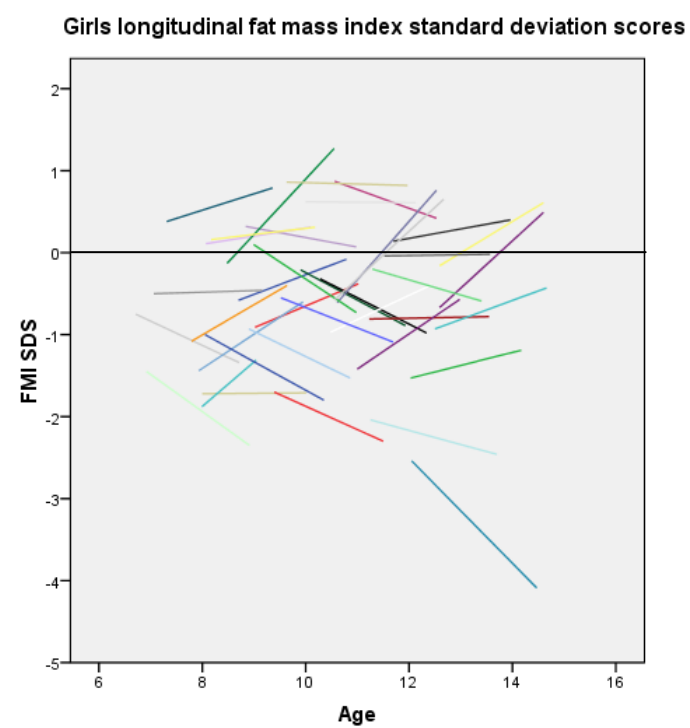
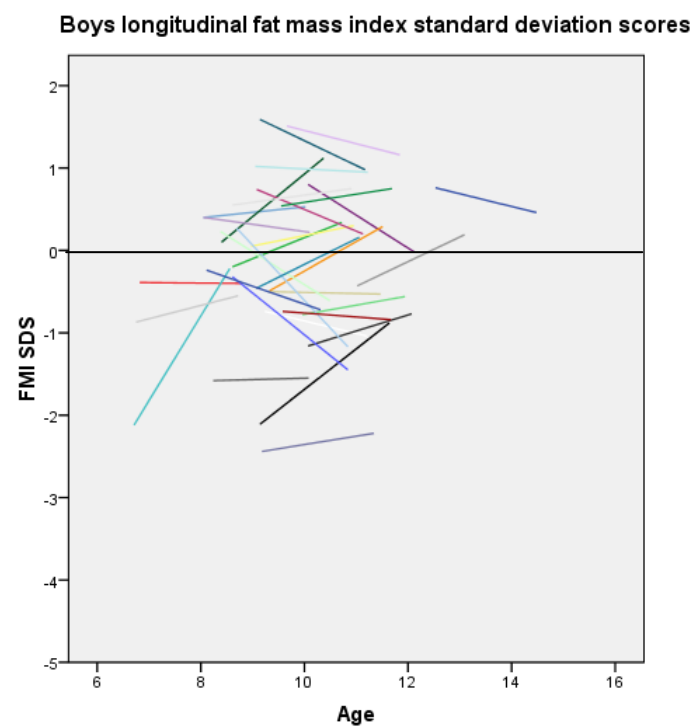
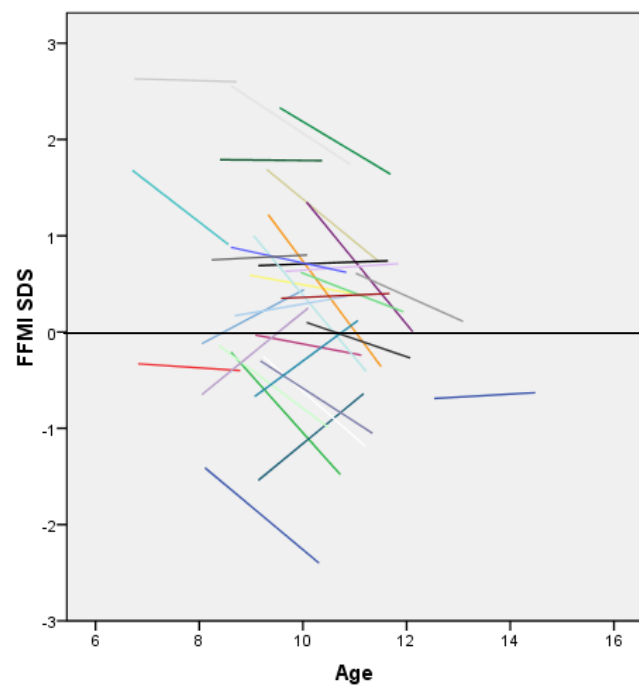


Figure 7.2. Individual change in fat mass index standard deviation score from baseline to two years

Boys longitudinal fat-free mass index standard deviation scores



Girls longitudinal fat-free mass index standard deviation scores

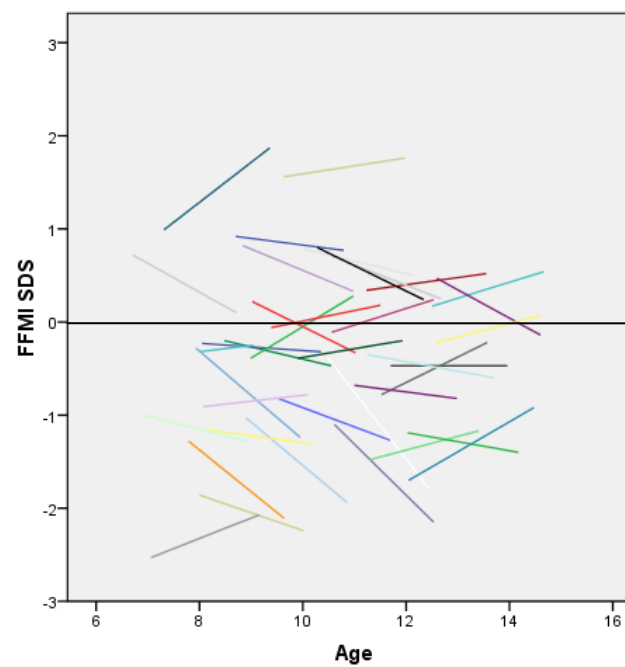


Figure 7.3. Individual change in fat-free mass index standard deviation score from baseline to two years

Table 7.3. Difference in change (cystic fibrosis (CF) minus control) between CF and control children.

Positive values denote larger change in children with CF

	Boys (CF 31, control 44)			Girls (CF38, control 49)		
	Mean	SEM	<i>P</i>	Mean	SEM	<i>P</i>
Age yrs	0.05	0.02	0.030	0.02	0.04	0.501
Weight SDS	-0.18	0.10	0.081	-0.04	0.08	0.562
Height SDS	0.03	0.07	0.959	-0.02	0.09	0.646
BMI SDS	-0.25	0.13	0.056	-0.07	0.09	0.502
Waist SDS	-0.25	0.10	0.022	0.07	0.11	0.638
LS BMD SDS	0.02	0.09	0.768	-0.14	0.14	0.271
LS BMAD SDS	0.17	0.14	0.456	0.06	0.13	0.135
FMI SDS	-0.06	0.15	0.698	0.08	0.12	0.464
FFMI SDS	-0.32	0.14	0.029	-0.26	0.10	0.017
PMI SDS	-0.41	0.24	0.093	-0.14	0.22	0.518
MMI SDS	-0.10	0.19	0.368	0.04	0.14	0.659
Sum4 skinfold thickness SDS	-0.23	0.13	0.091	0.11	0.12	0.352
Activity (hours)	-0.99	1.48	0.507	-1.37	0.90	0.157

Independent *t*-test, mean difference is CF minus control, which were as follows; boys, age difference (mean(SD), CF; 2.08(0.13), control; 2.03(0.06)), waist SDS, (CF; -0.20 (0.46), control; 0.05 (0.45)), body volume, (CF;6.25(3.07), control; 8.53(5.56)) and FFMI SDS, (CF;-0.35 (0.67), control; -0.05 (0.51)).

Difference in girls FFMI SDS was CF; -0.14 (0.53), control; 0.11 (0.46).

7.6.3 Predictors of conditional change in fat mass index, fat-free mass index and mineral mass index standard deviation scores

7.6.3.1 Choice of variables.

Conditional change in FMI, FFMI, and MMI SDS were chosen as the dependent variables (DV). Although MM is part of FFM it was included in the analysis because the girls with CF showed a deficit at baseline. It is more usual to assess bone mineralisation using BMD SDS obtained by DXA, however, MMI SDS from the 4CM is included in the analysis for consistency with FM and FFM. Initial regression analysis in girls suggested that FMI SDS predicts conditional change in FFMI SDS and FFMI SDS predicts conditional change in FMI SDS. However, if there is measurement error in FM there will be an equal and opposite error in FFM since they are both calculated using the 4CM and this may confound the analysis. Therefore, values of FFM from DXA were used to investigate predictors of 4C FM and DXA FM to investigate 4C FFM and MM.

Choice of independent variables to enter in a regression model was decided on the basis of correlation analyses presented in **Table 7.4** for children with CF.

Table 7.4. Correlation analyses of potential predictors of body composition variables in children with cystic fibrosis.

	Conditional FMI SDS				Conditional FFMI SDS				Conditional MMI SDS			
	Boys		Girls		Boys		Girls		Boys		Girls	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Weight SDS year 0		NS		NS		NS		NS		NS		NS
Conditional weight	0.60	<0.001	0.63	<0.001	0.58	<0.001		NS	0.40	<0.05	0.54	<0.01
Height SDS year 0		NS		NS		NS		NS		NS		NS
Conditional height		NS		NS		NS		NS	0.43	<0.05	0.46	<0.01
BMI SDS year 0		NS		NS		NS		NS		NS		NS
Conditional BMI SDS	0.63	<0.001	0.76	<0.001	0.57	<0.001		NS	0.36	<0.05		NS
Puberty year 0		NS		NS		NS		NS		NS		NS
Change in puberty		NS		NS		NS		NS		NS		NS
FMI SDS year 0		NS		NS		NS		NS		NS		NS
Conditional FMI SDS						NS		NS		NS		NS
FFMI SDS year 0		NS		NS		NS		NS		NS		NS
Conditional FFMI SDS		NS		NS					0.59	<0.001		NS
MMI SDS year 0		NS		NS		NS	0.36	<0.05		NS		NS
Conditional MMI SDS		NS		NS	0.59	<0.001		NS				
Activity year 0		NS		NS		NS		NS		NS		NS
Hours activity year 0		NS		NS		NS		NS		NS		NS
Change in hours activity		NS		NS		NS		NS		NS		NS
FEV ₁ SDS year 0		NS		NS	0.42	<0.05		NS	0.38	<0.05		NS
Conditional FEV ₁ SDS	0.41	<0.05		NS		NS		NS		NS		NS
Genotype		NS		NS		NS		NS		NS		NS

Correlation analysis is Pearson's for normally distributed and Spearman's rho for skewed data, puberty is classed as Tanner stage 1 (pre-pubertal) compared to stages 2-5. Change in puberty from baseline to 2years is; (1) Tanner stage 1, no change, (2) Change from stage 1, (3) no change from stage 2-5. Activity classed as; less than, same as or more than peers. Genotype is homozygous versus heterozygous $\Delta F508$ (48 v 17)

There was correlation between conditional weight and conditional BMI and the body composition variables as expected, since weight is the total of FM and FFM and $BMI = FMI + FFMI$. Weight and BMI were not included in the model. Although change in pubertal status did not show any correlation with the body composition variables in this sample, it was included in the regression analysis because pubertal development affects body composition. The independent variables included in the backward regression analysis were therefore;

Table 7.5. Dependent and independent variables used in regression analysis to identify predictors of body composition variables.

Independent variable	Dependent variable		
	Conditional change in FMISDS	Conditional change in FFMI SDS	Conditional change in MMI SDS
Baseline height SDS	Yes	Yes	Yes
Conditional change in height SDS	Yes	Yes	Yes
Baseline FMI SDS	Yes	Yes	Yes
Conditional change in FMI SDS		Yes	Yes
Baseline FFMI SDS	Yes	Yes	
Conditional change FFMI SDS	Yes		
Baseline MMI SDS			Yes
Conditional change MMI SDS			
Change in pubertal stage	Yes	Yes	Yes

In addition the analysis in children with CF included baseline and conditional change in FEV1 SDS.

7.6.3.2 Conditional change in body composition of children with cystic fibrosis.

Significant predictors from separate multiple backward regression analyses are presented in **Table 7.6** (boys) and **Table 7.7** (girls). Conditional change in height was a positive predictor of conditional change in MMI SDS in all children with CF. There were no identified predictors of conditional change in FMI or FFMI SDS in girls.

Table 7.6. Regression analysis of conditional change in; fat-mass index or fat-free mass index or mineral mass index standard deviation scores in boys with cystic fibrosis.

	Boys n=31		
	<i>B</i>	95% confidence interval	<i>P</i>
Conditional change in 4C FMI SDS (DV)			
Conditional change in FEV ₁ SDS	0.27	0.04, 0.51	0.024
Adjusted R ²	0.14		
Conditional change in 4C FFMI SDS (DV)			
DXA conditional change in FMI SDS	0.45	0.09, 0.80	0.016
FFMI SDS year 0	-0.23	-0.42, -0.05	0.016
Adjusted R ²	0.37		
Conditional change in 4C MMI SDS (DV)			
Conditional change in height SDS	0.69	0.21, 1.18	0.007
FEV ₁ SDS year 0	0.12	0.03, 0.21	0.013
Adjusted R ²	0.30		

Table 7.7. Regression analysis of conditional change in; fat-mass index or fat-free mass index or mineral mass index standard deviation scores in girls with cystic fibrosis.

Girls n=38			
<i>B</i>	95% confidence interval		<i>P</i>
Conditional change in 4C FMI SDS (DV)			
No significant predictors			
Conditional change in 4C FFMI SDS (DV)			
No significant predictors			
Conditional change in 4C MMI SDS (DV)			
Height SDS year 0	0.23	0.07, 0.39	0.007
Conditional change in height SDS	0.62	0.26, 0.99	0.001
MMI SDS year 0	-0.21	-0.38, -0.04	0.018
Adjusted R ²	0.40		

7.6.3.3 Absolute change in body composition of control children.

The group mean conditional change in the control group will have a value close to zero (because the regression equation is generated from the control data) and therefore finding factors that predict such a small value are unrealistic. In the control group I used regression analysis to investigate possible predictors of *absolute* change in FMI, FFMI and MMI SDS. None of the factors considered were significant for the body composition variables for boys, the data for girls is shown in (**Table 7.8**).

Table 7.8. Regression analysis of absolute change in; fat mass index or fat-free mass index or mineral mass index standard deviation scores in control girls.

	Girls n=49		
	<i>B</i>	95% confidence interval	<i>P</i>
Absolute change in 4C FMI SDS (DV)			
FMI SDS year 0	-0.13	-0.26, -0.003	0.046
DXA absolute change in FFMI SDS	-0.31	-0.60, -0.03	0.032
Adjusted R ²	0.16		
Absolute change in 4C FFMI SDS (DV)			
FFMI SDS year 0	-0.19	-0.33, -0.05	0.010
Adjusted R ²	0.14		
Absolute change in MMI SDS (DV)			
Height SDS year 0	0.17	0.06, 0.29	0.004
DXA FMI SDS year 0	0.11	0.01, 0.21	0.033
MMI SDS year 0	-0.15	-0.25, -0.05	0.003
Adjusted R ²	0.27		

7.6.4 Effect of cystic fibrosis on change in anthropometric and body composition variables

In order to determine whether having CF impacts on change in growth and body composition variables (height, weight, BMI, FMI, FFMI or MMI SDS) regression analyses were done with the 2 year measurement as the dependent variable, the baseline measurement as the covariant and condition (CF=1, control=0) as a fixed factor and the findings are presented in **Table 7.9**. Boys and girls were analysed separately. Condition was only significant for FFMI SDS in girls ($p<0.01$) and approached significance for BMI SDS in boys ($p=0.06$).

Table 7.9. General linear model for effect of condition (cystic fibrosis=1, control = 0) on growth and body composition

Girls (CF=38, control =49)			
	<i>B</i>	95% confidence interval	<i>P</i>
FFMI SDS year 2 (DV)			
FFMI SDS year 0	0.88	0.76, 0.99	<0.001
Condition 0	0.30	0.09, 0.50	0.006
Adjusted R ²	0.75		
Boys (CF=31, control =44)			
	<i>B</i>	95% confidence interval	<i>P</i>
BMI SDS year 2 (DV)			
BMI SDS year 0	1.01	0.86, 1.16	<0.001
Condition 0	0.25	-0.01, 0.51	0.060
Adjusted R ²	0.71		

Estimated means (SE) FFMI SDS in girls at 2 years after adjusting for baseline values was; CF -0.33(0.08) and controls -0.04 (0.07) and for BMI SDS at 2 years after adjusting for baseline values in boys; CF -0.08(0.10) and controls 0.17(0.08).

A separate general linear model was performed to test for any interaction between CF and baseline measurement of BMI SDS or FFMI SDS. There were no significant interactions.

7.6.5 Relationship between two-year change in body composition and two-year change in spirometry

Mean FEV₁ SDS was; CF boys, mean (SD), baseline -1.10 (1.54) and 2 years -1.15 (1.56) (Table 7.3) and CF girls, baseline -1.56 (1.44) and 2 years -2.05 (1.58) (Table 4). There was a significant deterioration in the girls' FEV₁ (p<0.01) between baseline and 2 years of 0.5 SDS. Boys and girls 2 year change in FEV₁ SDS compared to 2 year change in either FMI SDS or FFMI SDS is presented in **Figures 7.4 and 7.5**. Correlations between change in FEV₁ SDS and change in FMI SDS were boys; R = 0.51, p<0.01, girls; 0.12, NS and between change in FEV₁ SDS and change in FFMI SDS, boys; R = 0.07, NS, girls; -0.001, NS. Correlations between conditional change in FEV₁ SDS and conditional change in either FMI or FFMI SDS gave similar results.

a)

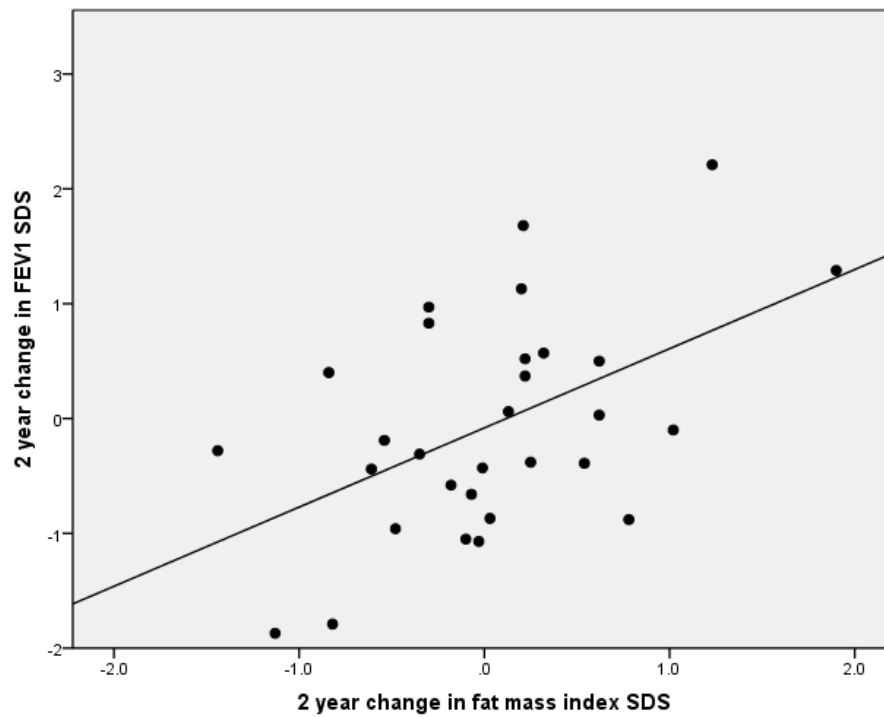
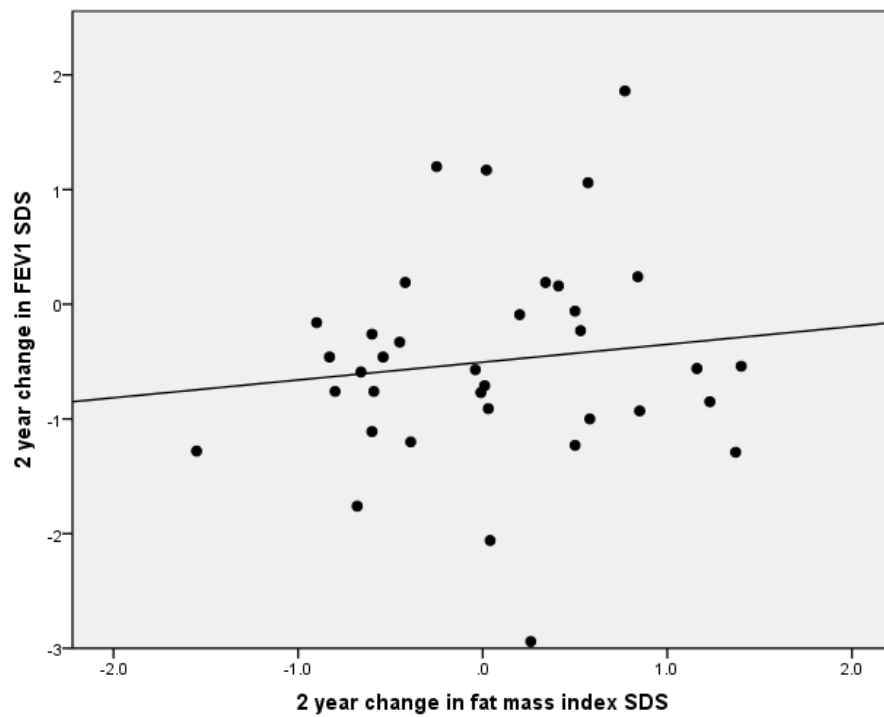


Figure 7.4 Two year change in FEV_1 and fat mass index SDS in a) boys and b) girls with cystic fibrosis.

b)



a)

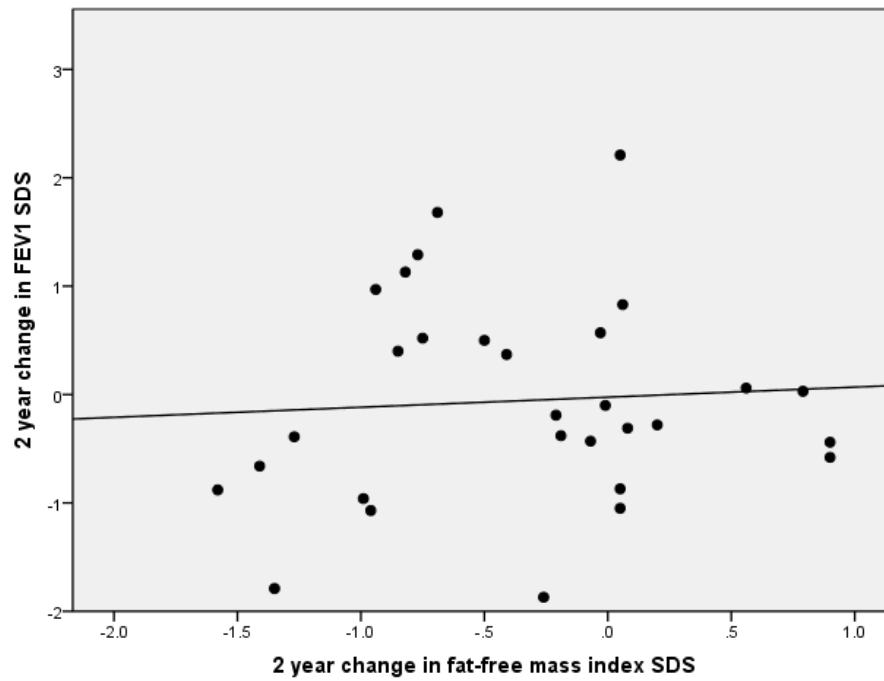
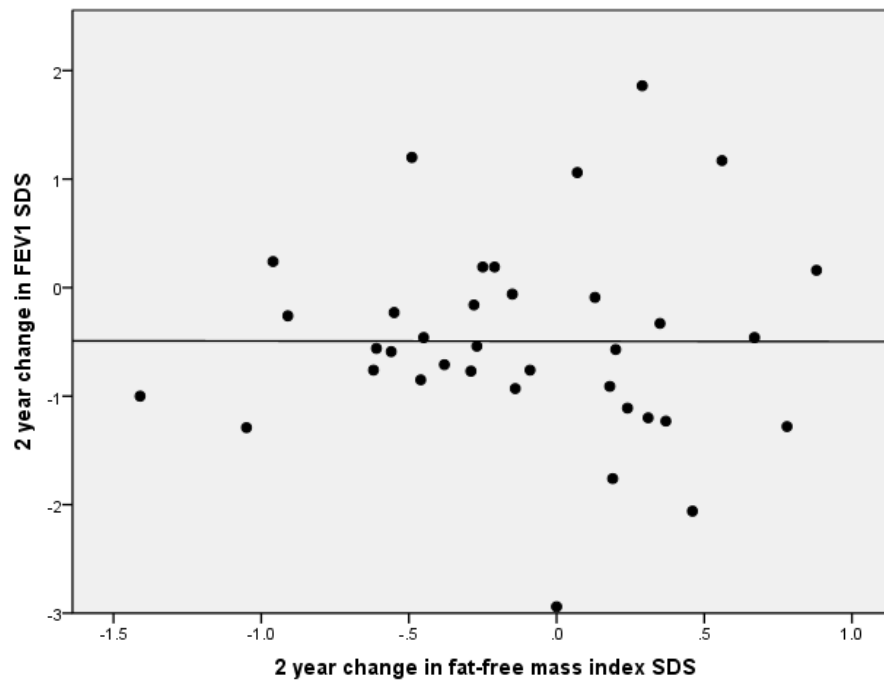


Figure 7.5 Two year change in FEV1 and fat-free mass index SDS in a) boys and b) girls with cystic fibrosis.

b)



In the analysis of factors predicting change in body composition variables conditional change in FEV₁ SDS was a significant predictor of conditional change in FMI SDS and baseline FEV₁ SDS predicted conditional change in MMI SDS in boys only. From a clinical perspective it is helpful to know if any body composition, anthropometric or other variables predict FEV₁ SDS. Significant correlations were therefore investigated and presented in **Table 7.10**.

Table 7.10. Correlation between potential predictors and absolute change in FEV₁ standard deviation scores in children with cystic fibrosis

	Boys		Girls	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Weight SDS Yr 0	-0.18	0.341	0.14	0.401
Conditional change in weight SDS	0.36	0.050	0.14	0.420
Height SDS Yr 0	-0.24	0.201	0.11	0.520
Conditional change in height SDS	0.22	0.236	0.20	0.232
BMI SDS Yr 0	-0.02	0.927	0.15	0.372
Conditional change in BMI SDS	0.28	0.139	0.04	0.805
FMI SDS Yr 0	-0.29	0.120	0.01	0.950
Conditional change in FMI SDS	0.47	0.009	0.12	0.493
FFMI SDS Yr 0	0.19	0.313	0.23	0.181
Conditional change in FFMI SDS	0.07	0.697	0.06	0.723
MMI SDS Yr 0	0.03	0.865	-0.10	0.549
Conditional change in MMI SDS	0.15	0.445	0.31	0.069
LS BMD SDS Yr 0	0.08	0.688	0.09	0.608
Conditional change in LS BMD SDS	0.24	0.209	0.25	0.144
LS BMAD SDS Yr0	0.27	0.152	0.07	0.691
Conditional change in LS BMAD SDS	0.29	0.123	0.10	0.580
4 skinfolds SDS Yr 0	-0.07	0.716	0.09	0.611
Conditional change in 4 skinfolds SDS	0.21	0.265	-0.02	0.910
Pre-pubertal Yr 0	-0.09	0.636	0.08	0.664
Pre-pubertal Yr 2	-0.12	0.522	0.002	0.990
Change in pubertal status	0.13	0.491	-0.05	0.761
Activity level Yr 0	0.36	0.054	0.16	0.351
Activity (hrs)	0.10	0.591	0.21	0.224
Conditional activity (hrs)	0.26	0.168	0.14	0.431
Genotype	-0.23	0.230	-0.03	0.879

Considering the significant associations above, a regression analysis with conditional change in FEV₁ SDS as the dependent variable and conditional change in weight

SDS, FMI SDS, MMI SDS and activity and baseline FEV₁ SDS and activity as the independent variables was performed in the sexes separately. Secondly, conditional change in FFMI SDS was substituted for MMI SDS in a separate analysis because many previous studies have identified a relationship between lean tissue and lung function. The significant predictors of conditional change in FEV₁ are presented in **Table 7.11**.

Table 7.11. Regression analysis of conditional change in FEV₁ standard deviation scores and potential predictors in children with cystic fibrosis.

Boys (n=31)			
<i>B</i>	95% confidence interval		<i>P</i>
Conditional change in FEV ₁ SDS (DV)			
Conditional change in FMI SDS	0.69	0.20, 1.17	0.007
Change in activity (hrs)	0.07	0.02, 0.13	0.014
Adjusted R ²	0.34		
Girls (n=38)			
<i>B</i>	95% confidence interval		<i>P</i>
Conditional change in FEV ₁ SDS (DV)			
Conditional change in MMI SDS	0.49	0.03, 0.96	0.037
Adjusted R ²	0.12		

However, in a clinical situation it is not practical to calculate conditional change and therefore absolute change is more likely to be used. The analysis was repeated with absolute change in FEV₁ as the dependent variable and baseline and change in weight, FMI SDS, MMI SDS (or FFMI SDS) and activity and baseline FEV₁ SDS as the independent variables (**Table 7.12**). No significant predictors were identified in girls.

Table 7.12. Regression analysis of absolute change in FEV₁ standard deviation scores and potential predictors in boys with cystic fibrosis.

Boys (n=31)			
	<i>B</i>	95% confidence interval	<i>P</i>
Absolute change in FEV₁ SDS (DV)			
Absolute change in FMI SDS	0.69	0.23, 1.15	0.004
Adjusted R ²	0.23		

The relationship between spirometry and body composition and how one influences the other is conceptualized in the following diagram.

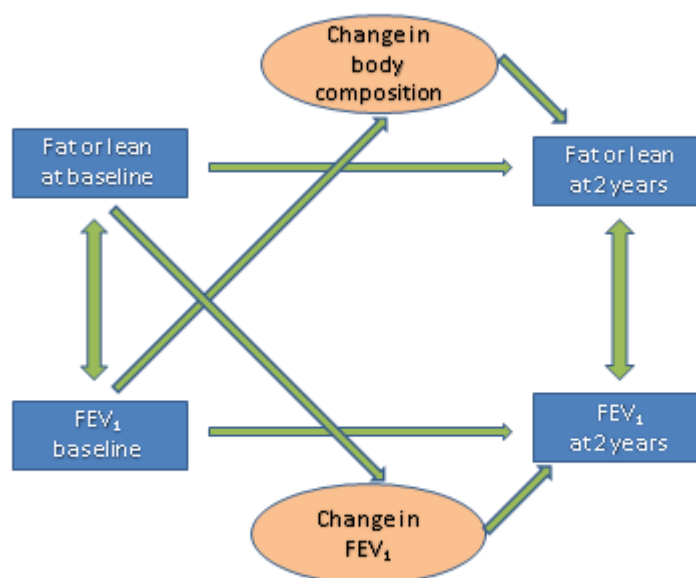


Figure 7.6. *Conceptual framework for the relationship between body composition and spirometry over time.*

I was interested to see to what degree, if any, baseline body composition SDS predicts change in FEV₁ SDS compared to the opposite situation where baseline FEV₁ SDS may predict change in body composition SDS. Using regression analysis with no adjustment I found no significant models of baseline FMI or FFMI SDS predicting change in FEV₁. For the opposite direction only baseline FEV₁ significantly predicted change in FFMI SDS in boys such that a 1 SDS higher FEV₁ at baseline predicted an additional 0.18 FFMI SDS change with a standard error of the mean of 0.07. Using conditional change in body composition and FEV₁ did not change the outcome. Data from the regression analyses may be found in **Appendix 10**. Therefore, in boys with CF change in FM predicts change in lung function and baseline lung function predicts change in FFM.

7.6.6 Relationship between activity and change in body composition

In this study physical activity was assessed in two ways; (1) as hours per week of vigorous activity (sports and physical education) and (2) as the parent's rating of their child compared to other children of the same age (less than, equal to or more than peers).

Table 7.13. Activity rating of children with cystic fibrosis

	Baseline				Year 2			
	Boys		Girls		Boys		Girls	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hours activity per week	8.10	4.29	8.13	4.65	9.21	5.19	6.76	3.48
Activity level								
Less than peers	12.9%		7.9%		22.6%		13.2%	
Equal to peers	48.4%		50.0%		29.0%		57.9%	
More than peers	38.7%		42.1%		48.4%		28.9%	

Baseline activity and change in activity did not have an association with change in any body composition variables. However, there was a correlation between activity level at 2 years and conditional change in FFMI SDS ($r=0.55$, $p<0.001$) and MMI SDS ($r=0.38$, $p<0.05$) in boys with CF only. Using a general linear model adjusting for pubertal status at 2 years and conditional change in FEV₁, the estimated marginal means for the relationship between activity level and conditional change in FMI SDS was 0.68 SDS difference between the most active and least active categories. The most active group have a less than expected change in FMI SDS than if they were growing according to the control group and the least active have a higher than expected gain in FMI SDS. For conditional change in FFMI SDS the most active have, on average 0.94 SDS difference from the least active and were tracking a centile compared to the healthy boys, whereas the least active have a less than expected change in FFMI SDS. There was no relationship between activity and change in body composition in the girls.

7.7 Discussion

In this analysis I have investigated changes in body composition variables and compared changes in children with CF and controls, and addressed the question of whether baseline value and changes in components of body composition are associated with clinical outcome as assessed by spirometry in children with CF. It is important to emphasise that it is not possible to determine cause and effect in my observational analysis for example, does improved lung function lead to an improvement in body composition or vice versa?

7.7.1 Body composition

When comparing patients to a group of control children one would hope that the controls have a 'normal' growth pattern. The controls in this study did not change SDS for body composition components however the boys increased in weight and SFT SDS and the girls in waist and SFT SDS. In accord with trends presented in other studies the boys with CF have a significant reduction in FFMI SDS over 2 years both in absolute terms and compared to that in control children. However some studies suggest that deficits occur later in adolescence (36;100). The use of simple, easily available measurements of anthropometry could lead to the conclusion that the girls with CF are fatter after 2 years because waist and SFT SDS increased. However, increases in waist SDS in children with inflammatory conditions cannot be assumed to be as a result of central fat deposition since distention of the abdomen may be caused by enlarged organs and/or oedema. The fact that 4C FM did not increase over this period, remaining significantly low, contradicts this supposition. The girls with CF were depleted in FFM and both boys and girls with CF show downward centile crossing compared to the controls. The fact that both boys and girls are not sustaining FFMI SDS over time is likely to be associated with declining lung function even at this young age (10;116) although this is only apparent at this time in the girls of this study. The literature reports declining lung function in both sexes (10;116), and it is likely that other factors also impact on lung function. The boys in this study perform more vigorous exercise than the girls and this may either

contribute to or be in response to their better lung function. The pattern of change in FFMI can be seen graphically in Figure 7.3; the graph for boys shows that most are showing a reduction or staying the same, only 4 show a good positive change. However, the age range of the boys is lower than that of the girls and the graph for girls indicates that many have declining or the same SDS till around age 11 and thereafter those with declining and those with improving SDS are about equal. Of the 7 girls with increasing FFMI SDS after age 11, 6 were classed at Tanner stage 2 or 3, of the 7 remaining stable or declining in FFM, 5 were Tanner stage 1 and 2 stage 2 at baseline. These findings are in accord with reports of normal growth patterns with increases in FFM, in particular MM in the later stages of puberty in girls (228). Despite the children with CF not maintaining FFMI SDS this is not due to declining MM.

In this study, conditional change in body composition was significantly associated with activity level at 2 years in CF boys, the most active having a difference of 0.68 FMI and 0.94 FFMI SDS compared to the least active group. Conditional SDS for FMI were negatively and for FFMI positively associated with activity level. Conditional change in body composition in girls was not associated with activity level in girls which may, in part, be due to the girls exercising at lower levels than boys. Mothers of boys with CF rated 48% as having greater physical activity than peers whereas only 29% of girls with CF were rated as higher. Indeed, the CF girls were also less active than control girls who maintain the time spent in vigorous activity from baseline to 2 years whereas the girls with CF had a mean reduction of 1 hour 22 minutes per week. In addition, the fact that the girls have poorer lung function may be a contributory factor to a reduction of vigorous physical activity. An objective measure of physical activity would be more accurate than parental assessment.

It is useful to identify any baseline measurement that predicts change so that treatment may be targeted appropriately. In addition, change over a period of time may predict future outcomes and be useful for clinicians. Original height and change in height above that expected are positive predictors of conditional change in MMI SDS in boys and girls. This is not surprising since the growth spurt experienced in mid-puberty is accompanied by a period of peak bone accretion. Typically children

with CF have delayed puberty which may, in some part, account for any deficit in MM, although surprisingly, the girls with CF in this study report similar numbers at Tanner stage 1 (26.3%) to control girls (28.6%) at aged 8-14 years. However, the percentage of girls in Tanner stage 3 and above is CF 32.3% and controls 40.8%, showing that there are more control girls in the mid-pubertal stage, a period during which peak growth occurs in girls (228).

For boys with CF, baseline FFMI SDS predicted change above the expected in FFMI SDS; those with the lowest baseline score showing most 'catch-up' growth (or least decline). In addition, a 1 SDS change in FEV₁ above that expected for the group was associated with a 0.27 SDS increase above the expected for FMI SDS. There were no predictors of conditional change in FFMI SDS despite FFM SDS in girls decreasing compared to the controls, however, a lower MMI SDS at baseline was associated with 'catch-up' (or less decline) in MM. The fact that low FFM at baseline in boys predicts the greatest 'catch-up' of SDS is encouraging since it is predominantly FFM that is acquired during normal growth and maturation in boys. Although girls lowest in FM did not show the most 'catch-up', those lowest in MM did show most 'catch-up'.

When using SDS in children who are tracking a centile there would be little change in scores and this may in part explain the lack of predictors of absolute change in body composition variables in the control boys. However, predictors of change in MMI SDS in control girls were similar to predictors of change in MMI SDS in girls with CF (although CF analyses were based on conditional change), those who were tallest at baseline had the greatest increase in MMI SDS and those with lowest MMI SDS at baseline had the greatest increase. Unlike the girls with CF, the higher the FMI SDS at baseline the higher the change in MMI SDS. It is likely that high FM is acting as a proxy for puberty since the post pubertal females are likely to have the more positive change in FM. Conversely, low FFMI SDS at baseline is associated with lowest change in FFM in control girls. Separate general linear models to examine the effect of having CF on change in body composition revealed that the CF girls are significantly different from the control girls in respect of FFM but for boys the only factor that approached significance was BMI SDS despite their reduction in FFM.

7.7.2 Spirometry

Gender differences in lung function in CF are a moot point with some studies reporting no difference (13;121;229) and others, greater decline in either males (67;111) or females (10;39). In this study girls, even at a young age had poorer lung function than boys and it deteriorated over two years. This may be a reflection of their sub-optimal body composition and is not surprising given the poorer prognosis in females and may, in some part be related to girls being less active than boys (121).

In a clinical situation it is difficult to calculate conditional change and absolute change is more likely to be considered. Using absolute change the only factor predicting FEV₁ SDS is change in FMI SDS in boys only with a 1 SDS change in FMI being associated with a 0.7 change in FEV₁ SDS. However, when conditional change in FEV₁ SDS is considered, that is change after removing the effect of the baseline measurement, a large change in activity of 10 hours per week is associated with a 0.7 increase in FEV₁ SDS in addition to the association with FM. There is a weak association between conditional change in FEV₁ SDS and conditional change in MMI SDS in girls. There are several reports of an association between FFM and lung function in the literature (65;230) and in particular bone (36;119) but only Ahmed et al (111) has found a relationship between FM and FEV₁ in boys, however, it is not clear whether this is a relationship between change in FM and change in lung function and only a crude prediction technique (SFT) had been used. There may be several explanations for the fact that in this study a relationship between change in FM and change in lung function was found in boys. I have used a 'criterion' technique whereas many studies use inappropriate techniques to predict FFM such as SFT (121;231), the age range is narrow, the sexes are analysed separately and an adjustment for short stature has been made. The differing findings dependent on whether absolute or conditional change are used are important when considering the outcome since a 1 SDS change in FEV₁ has a different impact dependent on the baseline value. In this chapter I have investigated the relationship between lung function and body composition but it is impossible to say to what degree one is a result of, or influences the other. A randomized controlled trial of a treatment that

either impacts on body composition or lung function would be necessary to investigate the relationship further. However, simple regression analyses to investigate whether either baseline body composition predicts change in FEV₁ or whether baseline FEV₁ predicts change in body composition revealed that only baseline FEV₁ SDS predicted change in FFMI SDS such that a 1 SDS higher FEV₁ predicted a 0.18 higher FFMI SDS at 2 years in this group of boys with CF. This of course does not prove cause and effect but is interesting in the debate about the relationship between lung function and body composition. In summary, in this group of boys with CF increased change in FM predicted increased change in lung function and baseline lung function predicted change in MM. Since those boys who were exercising the most at 2 years had better FFM it is possible that those with good lung function are more likely to exercise which is likely to impact positively on FFM.

7.8 Study limitations

Ideally, conditional change in FEV₁ SDS would be calculated by generating a regression equation in the healthy children. Unfortunately, the control children did not undergo lung spirometry, however, the conditional change calculated in the CF group represents change that is not associated with the baseline FEV₁.

Children with CF can become ill and quickly change in their appetite and exercise ability. When I asked about the typical amount of time spent in vigorous physical activity it is likely that, to some degree, the answer will be shaped by the child's current medical condition. I have examined change over 2 years by measuring the children at baseline and 2 years and therefore these measurements represent a 'snapshot' in time, with some children fluctuating in body composition, lung function and activity level and others remaining more stable. Without more frequent measurements which may be onerous to the child and family it is not possible to define the pattern of body composition in the interim.

There are many factors that impact on growth and body composition in children with CF. No account of nutritional intake, cortico-steroid use, lung infections and hospital admissions have been accounted for in this study. However, obtaining accurate

records of clinical details was difficult in some of these children who are managed predominantly by their local hospital paediatrician. The use of FEV_1 allows for an objective measure of 'health' although in some children, particularly the very young the results are dependent on the child's motivation. However, the use of FEV_1 as a clinical indicator is typical of many studies and allows for comparison.

7.9 Summary

In this chapter I have investigated change in body composition and lung function in children with CF over a 2 year period.

- Both boys and girls were downward centile crossing compared to control children in respect of FFM.
- There is an indication that FFM is increasing in some of the older girls in the later stages of puberty but this needs to be confirmed with larger numbers as the children become older.
- Boys who are low in FFM and girls who are low in MM at baseline show the most 'catch-up' (or least decline) in SDS. FFM in boys and MM in girls are the tissues that are predominantly acquired during puberty.
- Change above that expected for FM in boys and MM in girls was associated positively with a greater conditional change in lung function (more than the rest of the group). If absolute change is considered, only change in FM in boys is related to change in lung function which is contrary to the literature.
- Level of activity at baseline was not associated with later body composition but in boys with CF those rated most active at 2 years had a lower than expected gain in FM and a gain in FFM similar to controls whereas the least active at 2 years had a greater than expected gain in FM and lower than expected FFM.
- Baseline FEV_1 predicts change in FFMI SDS in boys.
- Lung function is poorer in girls and may reflect their sub-optimal body composition which is not surprising given the poorer prognosis in females.
- The fact that the girls exercise less than the boys may be one explanation for their deteriorating lung function or alternatively poorer lung function may be

affecting their ability to exercise. It is also likely that sub-optimal body composition has an impact on lung function and exercise ability.

Chapter 8. An assessment of clinical tools for measuring body composition in children with cystic fibrosis using standard deviation scores

8.1 Introduction

In Chapter 3 I discussed body composition techniques and the advantages and disadvantages of each technique. Only cadaver analysis may be considered a gold standard for body composition measurement and all in-vivo techniques do not measure body composition directly but predict it from the measurement of other body properties. In vivo techniques therefore suffer from 2 forms of error, methodological error in collecting the data and error from the theoretical assumptions used to convert the data to body composition components (142). The simple techniques make different assumptions which may affect their suitability in different conditions and therefore no one technique is appropriate for all subjects at all times. The 4CM is considered a criterion method because the assumptions made are minimal, merely that there is a constant relationship between osseous and non-osseous mineral. In this chapter I will assess the accuracy of the simple techniques compared to the 4CM using SDS for each technique derived from the same reference population when measuring children with CF.

Comparison of my data with those of previous studies is complicated by the various techniques used to assess body composition. Although all techniques suffer from assumptions about the nature of components of body composition, the simple prediction and 2CM methods rely heavily on assumptions about the nature of the FFM which, in children, is constantly changing with maturation and growth. Hydration, for example, which is highly influential on the outcome in the simpler models, is not constant across age and sex and does not change linearly from birth to adulthood. In addition, error is introduced if published constants are used to convert, for example, TBW to FFM, since in this example, the hydration factor is the mean value by age and sex that has been derived from healthy populations and therefore

assumes that the individual has an average hydration. Comparison between techniques is important for research purposes but also clinically because it is helpful to know whether the techniques are suitable in a given patient group or are interchangeable.

Using the same methods as those used in this study we have recently published reference data on body composition of 533 children and young people aged 4 to 22 which allows for the calculation of SDS for the 4CM and simpler techniques. In this chapter I will address the question of whether the same result is achieved using SDS regardless of the technique used and in addition, to compare longitudinal change in SDS by the different techniques.

I will also present the findings of a ‘wisdom of crowds’ (218) approach which postulates that by aggregating several independent predictions the resultant prediction will be more accurate and precise. If this is the case and if the prediction is comparable to, or better than that of, for example, a DXA scan it would allow much simpler, cheaper assessments of the body composition of children with CF and thereby aid clinical assessment. Even with less accuracy the approach may be useful when more sophisticated equipment is not available.

8.2 Study design

This is a cross-sectional study comparing the outcome of simple body composition techniques with that of the 4CM in SDS for all children recruited to the study and for all time-points. In addition, the longitudinal measurements by different techniques are compared in the same cohort as that presented in Chapter 7.

8.3 Recruitment and exclusion criteria

Children with CF were recruited from patients at Great Ormond Street Hospital for Children; details can be found in Chapter 4. In addition, for the cross-sectional

analyses a further 27 adolescents with CF who were part of another study using the same techniques were included to extend the age range.

8.4 Methods

Measures of anthropometry and BMC, TBW and densitometry for 4CM were performed as described previously.

8.5 Statistical analyses

Data for males and females and for every available time-point were pooled for purposes of the analysis. The accuracy of the simpler methods was assessed using the criterion 4CM as the reference method for adiposity (4C FM SDS) and leanness (4C FFM SDS). DXA FM, SFT and BMI SDS were used as simple measures of adiposity and DXA FFM, TBW and BIA (height^2/Z) SDS were used as simple measures of leanness.

8.5.1 Size adjustment and standard deviation score calculation

Characteristics of all the children were compared to 1990 UK reference data to generate SDS for weight, height, and BMI. SDS compared to our contemporary reference population for body composition variables were calculated for; (i) absolute values of 4C FM, 4C FFM, DXA FM, DXA FFM, DXA limb FM, DXA limb FFM, DXA trunk FM, DXA trunk FFM, TBW, BIA and SFT and (ii) size adjusted (described in Chapter 4) 4C FMI, 4C FFMI, DXA FMI, DXA FFMI, DXA limb FMI, DXA limb FFMI, DXA trunk FMI and DXA trunk FFMI. All SDS were calculated using the lmsGrowth program©(210).

Significance of the mean value from zero was tested using independent *t*-tests, and paired *t*-tests were used to compare the mean difference of the simpler methods with that of the 4CM.

8.5.2 Comparison of the simpler body composition techniques and the 4-component model

The method of Bland and Altman (217) was used to assess agreement between absolute values for FM by DXA (whole body and regional), SFT and BMI SDS with 4C FM SDS and for agreement between the absolute values for FFM by DXA (whole body and regional), TBW and BIA SDS with 4C FFM SDS. In addition, height adjusted FMI by DXA (whole body and regional) was assessed for agreement with 4C FMI and FFMI by DXA (whole body and regional) was assessed for agreement with 4C FFMI SDS. The mean difference between techniques (bias; simple method - 4CM) and the ± 2 SD of the difference between techniques (limits of agreement) were calculated. The bias was then tested for significance from zero by using an independent *t*- test. The extent to which the magnitude of the bias was related to the magnitude of the variable was calculated as the correlation between the difference and the mean of the measured values. Correlations were performed unadjusted, adjusted for age and adjusted for age and sex. All analyses were performed using Statistical Package for Social Sciences 18.0 (SPSS Inc., Chicago) and $p < 0.05$ was considered significant.

8.5.3 Predictors of bias

Backward regression analyses were used to identify significant predictors of the bias in FM(I) and FFM(I) SDS. Analyses were performed with age, sex and BMI SDS as explanatory variables with sex coded as male = 1 and female = 2.

8.5.4 Calculation of a correction factor for bio-electrical impedance

Preliminary analysis suggested that a correction factor could be applied to the BIA SDS to improve accuracy (see 8.6.3). I calculated a correction factor in 2 ways; firstly using regression analysis to investigate the relationship between bias in BIA (compared to 4CM) and BIA SDS in the whole group of BIA measurements (n=141) and using the value for the slope as the predicted average of the group. Each individual BIA SDS was then corrected as follows; average corrected BIA SDS = actual BIA SDS - predicted average bias in BIA SDS. Secondly, the relationship between bias in BIA SDS (compared to 4CM) and BIA SDS was investigated taking into consideration possible significant predictors of bias. I used backward linear regression with independent variables: age, sex (male=1, female=2), puberty (pre pubertal=1, post pubertal =2) and BMI SDS in a subset of measurements that were not used in the longitudinal analyses (n=60). The resultant equation was used to correct each individual's BIA SDS as follows; individual corrected BIA SDS = actual BIA SDS – predicted individual bias in BIA SDS.

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8.5.5 Longitudinal comparison

The ability of the simpler techniques to assess longitudinal change was compared to change assessed by the 4CM using SDS that have not been adjusted for height. Unadjusted (non-indexed) variables were chosen because all simple techniques apart from DXA do not generate FM or FFM SDS, rather SDS of that particular technique for example TBW SDS or SFT SDS and therefore it would be inappropriate to adjust for height. Two-year change in anthropometric and body composition SDS were compared to zero change using independent *t*-tests. Bland-Altman analyses were used to compare the bias in change (change by simple technique – change by 4CM) of body composition variables with limits of agreement of $\pm 2SD$. The degree to which the magnitude of the bias was related to the magnitude of the variable was assessed with correlation analyses.

8.5.6 Categorisation of ‘abnormal’ standard deviation scores

A ‘normal’ body composition SDS was classed as an SDS between -2 and +2, since these cut-offs are commonly used in clinical practice to define ‘normality’. The ability of each technique to categorise ‘normal’ and ‘abnormal’ body composition was assessed by cross-tabulation of the simple technique with the 4CM and calculation of Cohen’s kappa coefficient (κ) and % agreement. Whilst % agreement is a measure in absolute terms, Cohen’s kappa also takes into account the probability of agreement occurring by chance, a value of 1 indicating perfect agreement.

8.5.7 ‘Wisdom of crowds’ approach to determine whether aggregate predictions improve accuracy.

The ‘wisdom of crowds’ approach utilises the simplest, readily available prediction techniques and may be useful when more sophisticated techniques are not available. This approach is based on the theory that using many predictions will give an answer closer to the truth than using one or two predictions (a full explanation may be found in 4.6.8). To test this hypothesis in this particular population I used 12 different prediction equations based on height, weight, SFT and BIA to calculate FM for each child and then aggregated them to compare with the 4CM. Values of TBW or body density from some equations were converted to FFM using our published data on density and hydration (184). FM was calculated as the difference between FFM and weight and vice versa. Only equations that spanned at least 6 of the 6-17yrs of those subjects in this study were selected although most covered the whole range. Only one equation was specifically derived for children with CF (Johnston). Only children with a value for FM from the 12 separate equations were used in the analysis, the number being reduced by lack of BIA data in several and the inability to calculate some predictions in the younger children for whom the equations were not appropriate.

8.6 Results

8.6.1 Subjects

Data from all children measured from 2001 to 2011 in this study and another of older children with CF measured using the same techniques, was included in the analysis. Of the 127 children enrolled, 81 children had subsequent re-measurements of between 1 and 5 times. 28 measurements were not included due to inadequate data or failed 4CM. Characteristics of the children are shown in **Table 8.1**, the age range was 6.7 to 17 years and the mean value for the group by sex indicates that they are both short and light for their age. Girls with CF had significantly low values for all body composition variables by all methods apart from having significantly high mean DXA trunk FFMI. FMI and FFMI SDS by 4CM were not different from zero in boys with CF although 4C FM and FFM SDS (unadjusted for height) and many of the simpler techniques suggest they had a depletion of both FM and FFM with the exception of higher trunk FMI and FFMI SDS by DXA.

8.6.2 Accuracy of simpler body composition techniques compared to the 4-component model

The simpler body composition techniques were compared to 4C FM and FFM in terms of mean bias and limits of agreement in individuals. **Table 8.2** and **Figure 8.1** (measures of ‘fatness’) and **Figure 8.2** (measures of leanness) summarise the mean bias and limits of agreement for each method. Graphs showing the individual biases may be found in **Appendix 12**.

Table 8.1. Anthropometry and body composition standard deviation scores by sex

Measurement method SDS ¹	Boys					Girls				
	N	Mean	SD	<i>P</i> ^{Ref}	<i>P</i> ^{4CM}	N	Mean	SD	<i>P</i> ^{Ref}	<i>P</i> ^{4CM}
Age (y)	124	11.8	2.68			142	11.9	2.63		
Weight	124	-0.23	1.10	0.023		142	-0.57	1.18	<0.001	
Height	124	-0.52	1.08	<0.001		142	-0.57	1.14	<0.001	
<i>Measure of 'fatness'</i>										
BMI	124	0.10	1.07	0.324	<0.001	142	-0.32	1.09	0.001	<0.001
4C FM	124	-0.32	1.00	0.001		142	-0.75	1.09	<0.001	
4C FMI	124	-0.09	1.18	0.108		142	-0.57	1.05	<0.001	
DXA FM	124	-0.29	0.92	0.001	0.394	142	-0.73	0.95	<0.001	0.521
DXA FMI	124	-0.05	0.90	<0.001	0.264	142	-0.46	0.93	<0.001	0.307
DXA limb FM	124	-0.58	1.83	0.001	0.033	142	-1.45	1.91	<0.001	<0.001
DXA limb FMI	124	-0.36	1.81	0.027	<0.001	142	-1.20	1.82	<0.001	<0.001
DXA trunk FM	124	0.02	0.91	0.983	<0.001	142	-0.42	0.95	<0.001	<0.001
DXA trunk FMI	124	0.14	0.89	0.081	<0.001	142	-0.28	0.92	<0.001	<0.001
Bicep SFT	123	-0.02	0.86	0.818	<0.001	142	-0.23	0.85	0.002	<0.001
Tricep SFT	124	-0.02	0.95	0.829	<0.001	141	-0.46	0.96	<0.001	<0.001
Subscapular SFT	124	-0.05	0.98	0.539	<0.001	142	-0.28	1.05	0.002	<0.001
Supra-iliac SFT	123	0.07	0.98	0.464	<0.001	134	-0.31	1.07	0.001	<0.001
Sum 4 SFT	122	-0.01	0.85	0.865	<0.001	133	-0.32	0.87	<0.001	<0.001

¹ Standard deviation score except for age, *P*^{Ref}; independent *t*-test for difference from zero, CF compared to reference data (54;155;209), *P*^{4CM}; paired *t*-test simple technique compared to 4-component model, BMI; body mass index, 4C; 4-component, FM; fat mass, FMI; fat mass index (FM/height²), DXA; dual-energy X-ray absorptiometry, SFT; skinfold thickness. **Table continues on next page.**

Table 8.1 continued. Anthropometry and body composition standard deviation scores by sex

	Boys					Girls				
Measurement method SDS ¹	N	Mean	SD	P^{Ref}	P^{4CM}	N	Mean	SD	P^{Ref}	P^{4CM}
<i>Measure of 'leanness'</i>										
4C FFM	124	-0.44	1.09	<0.001		142	-0.83	1.21	<0.001	
4C FFMI	124	0.07	1.13	0.482		142	-0.39	1.00	<0.001	
DXA FFM	124	-0.85	1.10	0.527	<0.001	142	-1.36	1.16	<0.001	<0.001
DXA FFMI	124	-0.09	1.18	0.406	<0.001	142	-0.57	1.05	<0.001	<0.001
DXA limb FFM	124	-0.85	1.10	<0.001	<0.001	142	-1.36	1.16	<0.001	<0.001
DXA limb FFMI	124	-0.70	1.06	<0.001	<0.001	142	-1.21	1.11	<0.001	<0.001
DXA trunk FFM	124	-0.09	1.14	0.374	<0.001	142	-0.40	1.09	<0.001	<0.001
DXA trunk FFMI	124	0.65	1.21	<0.001	<0.001	142	0.28	0.94	0.001	<0.001
TBW	124	-0.48	1.09	<0.001	0.075	142	-0.84	1.13	<0.001	0.580
BIA	65	-0.44	1.05	0.001	0.001	76	-1.16	1.21	<0.001	<0.001

¹ Standard deviation score except for age, P^{Ref} ; independent t -test for difference from zero, CF compared to reference data, P^{4CM} ; paired t -test simple technique compared to 4-component model, 4C; 4-component, FFM; fat-free mass, FFMI; fat-free mass index (FFM/height²), DXA; dual-energy X-ray absorptiometry, TBW; total body water, BIA; bio-electrical impedance.

Table 8.2. Bland-Altman analysis of mean bias in fat mass and fat-free mass standard deviation scores by simple body composition techniques compared to the 4-component model.

Measurement method SDS	N	Mean bias ¹	95% Limits of agreement	P^{bias}	R	$P^{\text{correlation}}$
<i>Measure of 'fatness'</i>						
DXA fat mass	266	0.15	±0.71	<0.001	-0.279	<0.001
DXA fat mass index	266	0.13	±0.71	<0.001	-0.251	<0.001
DXA limb fat mass	266	0.02	±0.74	0.295	-0.294	<0.001
DXA limb fat mass index	266	-0.001	±0.80	0.958	-0.233	<0.001
DXA trunk fat mass	266	0.16	±0.37	<0.001	-0.282	<0.001
DXA trunk fat mass index	266	0.16	±0.42	<0.001	-0.228	<0.001
Bicep skinfold	265	0.42	±1.35	<0.001	-0.331	<0.001
Tricep skinfold	265	0.29	±1.33	<0.001	-0.142	0.021
Subscapular skinfold	266	0.37	±1.32	<0.001	-0.072	0.245
Supra-iliac skinfold	257	0.42	±1.39	<0.001	0.001	0.982
Sum 4 skinfold	255	0.38	±1.04	<0.001	-0.350	<0.001
BMI	266	0.42	±1.42	<0.001	0.049	0.429

¹ Bias is the difference (simple technique – 4-component model (4CM)), 95% limits of agreement ; ±2SD, DXA; dual-energy X-ray absorptiometry, BMI; body mass index, P^{bias} ; independent t -test compared to zero, R ; Pearson's correlation, $P^{\text{correlation}}$; paired sample t -test between the bias and the mean of simple technique and 4CM.

Table 8.2 continued. Bland-Altman analysis of mean bias in fat mass and fat-free mass standard deviation scores by simple body composition techniques compared to the 4-component model.

Measurement method SDS	N	Mean bias ¹	95% Limits of agreement	P^{bias}	R	$P^{\text{correlation}}$
<i>Measure of 'leanness'</i>						
DXA fat-free mass	266	-0.11	±0.67	0.002	0.209	0.001
DXA fat-free mass index	266	-0.17	±0.88	<0.001	0.114	0.064
DXA limb fat-free mass	266	-0.47	±0.80	<0.001	0.096	0.121
DXA limb fat-free mass index	266	-0.80	±1.26	<0.001	0.049	0.424
DXA trunk fat-free mass	266	0.20	±0.39	<0.001	0.004	0.947
DXA trunk fat-free mass index	266	0.31	±0.63	<0.001	-0.002	0.977
Total body water	266	-0.02	±0.46	0.111	0.014	0.819
Bio-electrical impedance	141	-0.24	±0.92	<0.001	0.003	0.975
Corrected bio-electrical impedance ²	141	0.41	±0.92	<0.001	0.003	0.975
Corrected bio-electrical impedance ³	81	0.001	±0.80	0.986	-0.055	0.629
Combined DXA and BIA	141	-0.07	±0.53	0.560	-0.082	0.337

¹ Bias is the difference (simple technique – 4-component model (4CM)), 95% limits of agreement; ±2SD, DXA; dual-energy X-ray absorptiometry, BMI; body mass index, ²Corrected BIA SDS; BIA SDS – predicted average bias of the group calculated by regressing BIA SDS on bias BIA SDS, ³Corrected BIA SDS; BIA SDS – predicted bias calculated by regressing BIA SDS on bias BIA SDS adjusting for age and BMI SDS, P^{bias} ; independent t -test compared to zero, R ; Pearson's correlation, $P^{\text{correlation}}$; paired sample t -test between the bias and the mean of simple technique and 4CM.

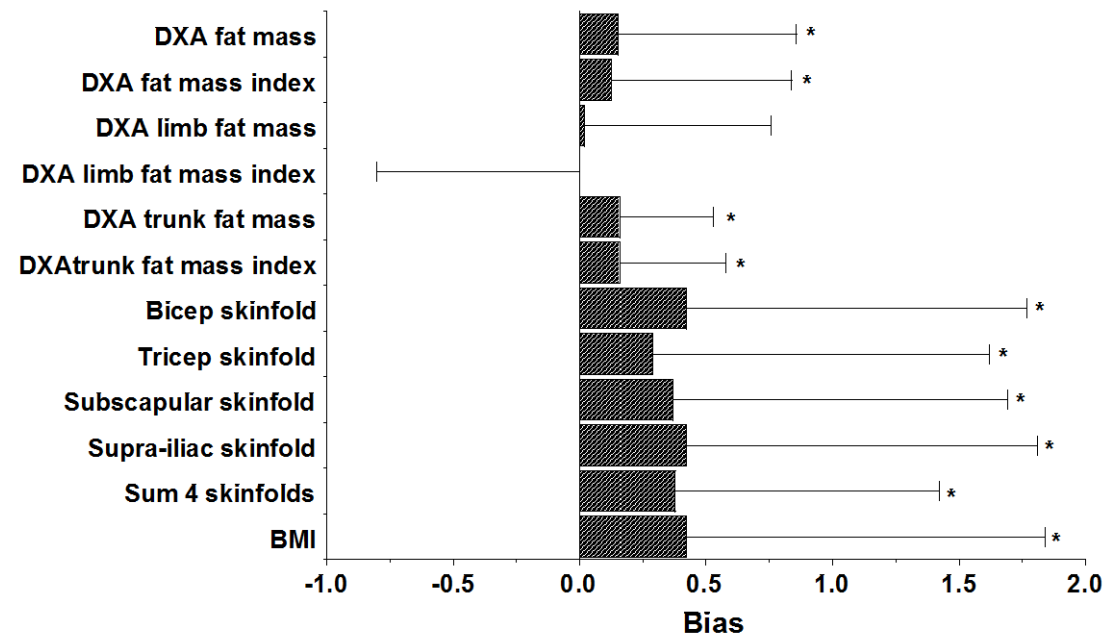


Figure 8.1. Bias and limits of agreement ($\pm 2SD$) for 'fatness' by simple techniques compared to the 4-component model (4CM; (simple technique SDS – 4CM FM SDS). All values are SDS, DXA; dual-energy X-ray absorptiometry, BMI; body mass index. Asterisks denote biases that were significantly different from the 4CM; * $p < 0.001$.

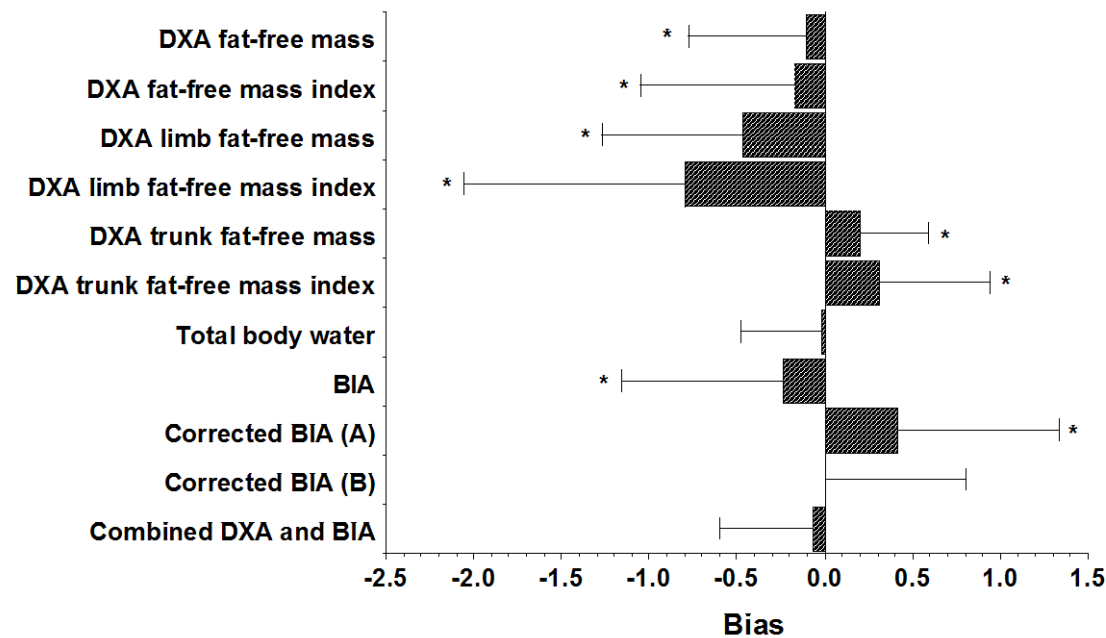


Figure 8.2. Bias and limits of agreement ($\pm 2SD$) for 'leanness' by simple techniques compared to the 4-component model (4CM; simple technique SDS – 4CM FFM SDS). All values are SDS, DXA; dual-energy X-ray absorptiometry, BIA; bio-electrical impedance. Asterisks denote biases that were significantly different from the 4CM, * $p < 0.005$.

8.6.2.1 Measures of 'fatness'

Compared to 4C FM SDS, DXA provided the most accurate measurement with lowest limits of agreement with a mean whole body bias of 0.15 ($p < 0.001$) and limits of agreement of ± 0.71 . However, although DXA trunk showed a similar bias (0.16 ± 0.37 , $P < 0.001$), DXA limb FM SDS showed insignificant bias from the 4CM (0.02) with limits of agreement of ± 0.74 . Children with CF tend to be short for their age and therefore the relationship between height-adjusted DXA and 4C FMI SDS was also examined and the results were similar.

BMI significantly overestimated FM SDS with wide limits of agreement (0.42 ± 1.42 , $p < 0.001$). All SFT SDS showed a significant bias and wide limits of agreement, ($0.29-0.42 \pm 1.32-1.39$). The mean of 4 SFT SDS improved the limits of agreement slightly but still showed a large significant bias (0.38 ± 1.04).

8.6.2.2 Measures of 'leanness'

Measurement of FFM SDS by DXA showed a small significant bias, underestimating by, on average 0.11 SDS with limits of agreement of ± 0.67 . Regional DXA FFM SDS gave a greater significant bias which was positive for trunk FFM SDS (mean bias $\pm 2SD$, 0.20 ± 0.39) and negative for limb FFM SDS (-0.47 ± 0.80). Adjusting DXA FFM for height compared to 4C FFMI SDS increased the bias and limits of agreement.

Compared to 4C FFM, TBW SDS provided the most accurate and precise value of FFM SDS, with an insignificant bias and narrow limits of agreement (-0.02 ± 0.46). When compared to the 4CM, BIA SDS significantly underestimated FFM SDS by 0.24 SDS and with limits of agreement of ± 0.92 .

Bland-Altman correlations were used to test whether the bias for each technique is related to the magnitude of the mean FM and FFM (Table 8.2). For DXA FM, bicep SFT, sum of 4 SFT and DXA FFM there was a highly significant correlation ($p < 0.001$). The correlation was less but significant for tricep SFT. For all measures

of lean except DXA whole body FFM there was no correlation between the magnitude of FFM and the bias. When age and sex were accounted for there was no difference in the findings (**Appendix 13**).

8.6.3 Predictors of the bias in fat mass and fat-free mass standard deviation scores

Multiple regression analyses were used to identify significant predictors of the bias in FM and FFM SDS (**Table 8.3**). Possible predictors considered were age, gender, pubertal stage and BMI. Pubertal stage (pre-pubertal v pubertal) was not a significant factor of bias for any technique. The predictive value of the models in explaining the bias was generally low with the adjusted R^2 not exceeding 0.19 except in the case of DXA FMI and DXA limb FM where the predictive value was 72% and 68% respectively. There was no single variable that was consistently the most significant predictor of bias across all measurement techniques. Age was significantly positively associated with DXA FMI, limb FM and BIA biases and negatively associated with bicep and tricep SFT and DXA whole body and trunk FFMI biases. The association of gender with bias was variable indicating no consistent trend for a bias in either FM or FFM in either sex. BMI showed a significant positive association with DXA whole body FMI, limb FM and negative association with bicep and tricep SFT and DXA trunk FFM(I).

BIA is a readily available simple technique that, although biased compared to 4C FFM SDS, displays no relationship in the data between the magnitude of the bias and magnitude of SDS. This allows for the calculation of a correction factor which may be calculated by adjusting each individual by the mean bias for the group or, considering the fact that age is a significant predictor of bias, taking into account possible predictors. Regression analysis between bias in BIA (compared to 4CM) and BIA SDS in the whole group of BIA measurements (n=141) gave a value for the slope of -0.173 which was used as the mean value correction factor for each individual's BIA SDS. When the corrected BIA SDS was compared to 4C FFM SDS

there was an increase in the bias compared to uncorrected BIA SDS. Secondly, the relationship between bias in BIA SDS and BIA SDS was investigated taking into consideration possible significant predictors of bias in 61 children whose data was not included in the longitudinal analysis. Only age and BMI SDS were significant and included in the final equation for prediction of bias which was tested in the remaining 81 measurements. The standard error of the estimate was calculated for the model to indicate accuracy in individuals. The resultant equation was;

$$\text{Predicted bias in BIA SDS} = -0.783 + 0.155(\text{BIA SDS}) + 0.058(\text{age}) - 0.176 (\text{BMI SDS})$$

Equation 8.1

This model has a standard error of the estimate of 0.44 SDS and explains only 20% of the variation in bias in BIA SDS. Using the above equation a correction was made in the remaining 81 measurements (Table 8.2) and there was no significant bias compared to the 4CM for the corrected BIA SDS values. The characteristics of the group with no BIA data, those used to generate the equation and those used to test the equation can be found in **Appendix 14**.

Mindful of the ‘wisdom of crowds’ approach I was interested to see if combining DXA and corrected BIA would give a lower bias and limits of agreement than for each technique separately and therefore I calculated the mean SDS $(\text{DXA} + \text{BIA})/2$ and compared that to the 4CM (Table 8.2). The bias of the combined techniques remained similar to that of corrected BIA and improved compared to the DXA bias but more importantly the limits of agreement were reduced to ± 0.53 in the combined SDS as opposed to DXA (± 0.67) and BIA (± 0.80) separately.

Table 8.3. Predictors of bias for different simple body composition measurements of (a) fat mass SDS and (b) fat-free mass SDS compared to the 4-component model.

(a) Bias compared to 4-component fat mass

Measurement method SDS	Age			Sex			BMI SDS			Adjusted R ²
	B	95%CI	P	B	95%CI	P	B	95%CI	P	
Measure of 'fatness'										
DXA fat mass	0.005	-0.011, -0.022	0.512	-0.011	-0.099, 0.077	0.809	-0.023	-0.063, 0.017	0.261	-0.004
DXA fat mass index	0.035	0.013, 0.058	0.002	-0.115	-0.237, 0.008	0.066	0.714	0.658, 0.770	P<0.001	0.718
DXA limb fat mass	0.039	0.015, 0.064	0.002	-0.145	-0.278, -0.012	0.033	0.713	0.652, 0.773	P<0.001	0.684
DXA limb fat mass index	-0.013	-0.031, 0.005	0.162	0.063	-0.035, 0.161	0.208	-0.031	-0.076, 0.013	0.168	0.011
DXA trunk fat mass	0.007	-0.003, 0.016	0.179	-0.004	-0.056, 0.047	0.870	-0.020	-0.043, 0.004	0.101	0.008
DXA trunk fat mass index	0.007	-0.003, 0.016	0.168	0.031	-0.109, 0.173	0.241	-0.008	-0.031, 0.016	0.533	0.005
Bicep skinfold	-0.034	-0.062, -0.006	0.017	0.117	-0.032, 0.267	0.124	-0.256	-0.324, -0.187	P<0.001	0.191
Tricep skinfold	-0.035	-0.064, -0.005	0.022	-0.072	-0.231, 0.087	0.373	-0.161	-0.233, -0.088	P<0.001	0.068
Subscapular skinfold	-0.029	-0.059, 0.001	0.058	0.190	0.029, 0.350	0.021	-0.043	-0.116, 0.031	0.255	0.029
Supra-iliac skinfold	-0.003	-0.036, 0.029	0.842	0.026	-0.148, 0.200	0.771	-0.059	-0.139, 0.020	0.144	-0.002
Sum 4 skinfold	-0.023	-0.046, 0.001	0.061	0.074	-0.053, 0.200	0.252	-0.122	-0.179, -0.064	P<0.001	0.073
BMI	-0.046	-0.096, 0.003	0.065	-0.410	-0.674, -0.152	0.002				0.041

Each line gives the results for a different multiple regression model. Sex coded as male=1, female=2

Table 8.3 continued

(b) Bias compared to 4-component fat-free mass

Measurement method SDS	Age			Sex			BMI SDS			<i>Adjusted R²</i>
	<i>B</i>	<i>95%CI</i>	<i>P</i>	<i>B</i>	<i>95%CI</i>	<i>P</i>	<i>B</i>	<i>95%CI</i>	<i>P</i>	
<i>Measure of ‘leanness’</i>										
DXA fat-free mass	0.005	-0.010, 0.021	0.492	-0.025	-0.108, 0.058	0.549	0.003	-0.035, 0.041	0.873	-0.008
DXA fat-free mass index	-0.024	-0.044, -0.004	0.019	-0.020	-0.127, 0.088	0.718	0.001	-0.042, 0.056	0.767	0.012
DXA limb fat-free mass	0.004	-0.014, 0.022	0.663	-0.102	-0.201, -0.004	0.041	0.028	-0.017, 0.073	0.225	0.015
DXA limb fat-free mass index	-0.002	-0.031, 0.028	0.911	-0.038	-0.195, 0.119	0.635	0.017	-0.055, 0.089	0.636	-0.009
DXA trunk fat-free mass	-0.003	-0.012, 0.005	0.444	0.016	-0.030, 0.063	0.488	-0.047	-0.068, -0.026	<0.001	0.064
DXA trunk fat-free mass index	-0.025	-0.039, -0.011	<0.001	0.014	-0.059, 0.088	0.699	-0.079	-0.113, -0.045	<0.001	0.098
Total body water	0.009	-0.002, 0.019	0.115	0.023	-0.034, 0.080	0.435	-0.003	-0.029, 0.023	0.803	0.002
Bio-electrical impedance	0.060	0.033, 0.088	<0.001	-0.091	-0.238, 0.056	0.221	-0.037	-0.104, 0.030	0.278	0.122

Each line gives the results for a different multiple regression model. Sex coded as male=1, female=2.

8.6.4 Comparison of longitudinal change in body composition by the simpler techniques compared to change assessed by the 4-component model

The group mean increased by (mean \pm SD); 0.20 \pm 0.67, (p=0.016) SDS for 4C FM and decreased by 0.13 \pm 0.50, (p=0.048) SDS for 4C FFM in the 2 year period (**Table 8.4**). The increase in SDS for 4C FM was replicated most closely by DXA trunk FM (0.17 \pm 0.53, p= 0.01) and the decrease in FFM SDS by 4CM was also identified by whole body and regional DXA FFM SDS and TBW SDS.

To investigate the difference between the simpler techniques and the 4CM for quantifying change in SDS a Bland-Altman analysis was performed (**Table 8.5** and **Figures 8.3** and **8.4**) with bias (change by simple technique SDS – change by 4C SDS), mean ([simple technique SDS + 4C]/2) and limits of agreement of ± 2 SD.

BMI, bicep SFT and uncorrected BIA SDS were the only techniques showing a significant bias for measuring change compared to the 4CM. Corrected BIA SDS had insignificant bias. Limits of agreement for measuring change with DXA were wide; 1SDS for FM and 0.6 SDS for FFM, although combining DXA and corrected BIA SDS improved the limits of agreement compared to DXA on its own from 0.6 SDS to 0.3 SDS. The magnitude of the bias was significantly inversely related to change in FM for BMI and DXA FM and there was a positive relationship between the bias and the magnitude of change in TBW SDS.

Table 8.4. Baseline measurements and change in longitudinal measurements using standard deviation scores

Measurement method SDS ¹	N	Baseline		Two year change				
		Mean	SD	Mean	SD	Range		<i>P</i> ^{change-zero}
Age (y)	69	9.34	1.55	2.08	0.13	1.84	2.50	<0.001
Weight	69	-0.33	1.06	-0.02	0.45	-1.41	1.24	0.677
Height	69	-0.55	1.07	0.08	0.38	-1.15	0.96	0.106
<i>Measures of 'fatness'</i>								
4CM fat mass	69	-0.64	0.99	0.20	0.67	-1.53	1.98	0.016
DXA fat mass	69	-0.49	0.92	0.13	0.54	-1.19	1.33	0.058
DXA limb fat mass	69	-0.60	0.91	0.14	0.54	-0.95	1.28	0.033
DXA trunk fat mass	69	-0.35	0.92	0.17	0.53	-1.20	1.27	0.010
Bicep skinfold thickness	69	-0.09	0.78	0.03	0.74	-1.90	2.10	0.723
Tricep skinfold thickness	68	-0.30	0.99	0.14	0.68	-1.40	1.60	0.104
Subscapular skinfold thickness	69	-0.21	0.95	0.07	0.81	-1.70	2.70	0.467
Supra-iliac skinfold thickness	66	-0.28	1.04	0.26	0.70	-1.50	2.60	0.003
Sum 4 skinfold thickness	65	-0.24	0.33	0.15	0.61	-1.30	1.55	0.193
BMI	69	-0.01	0.94	-0.09	0.54	-1.37	1.23	0.157
<i>Measures of 'leanness'</i>								
4C fat-free mass	69	-0.50	1.08	-0.13	0.50	-1.21	1.01	0.048
DXA fat-free mass	69	-0.61	1.13	-0.15	0.55	-1.78	1.38	0.039
DXA limb fat-free mass	69	-1.01	1.11	-0.08	0.50	-1.46	1.08	0.166
DXA trunk fat-free mass	69	-0.07	1.10	-0.17	0.61	-1.93	1.44	0.027
Total body water	69	-0.56	1.16	-0.09	0.60	-1.25	1.89	0.029
Bio-electrical impedance	32	-0.77	1.08	0.13	0.55	-0.92	1.32	0.193
Corrected bio-electrical impedance	32	-0.38	1.01	0.03	0.51	-1.01	1.15	0.739

¹ Standard deviation score apart from age, 4CM; 4-component model, DXA; dual-energy X-ray absorptiometry, BMI; body mass index, *P*^{change - zero}; paired *t*-test of change compared to zero, corrected bio-electrical impedance is individual bias in BIA SDS corrected for age and BMI SDS.

Table 8.5. Bias and limits of agreement of simpler methods compared to the 4-component model for change in fat mass and fat-free mass standard deviation scores.

Measurement method SDS	N	Bias ¹	95% Limits of agreement	P^{bias}	R	$P^{\text{correlation}}$
<i>Measures of 'fatness'</i>						
BMI	69	-0.29	±0.94	<0.001	-0.317	0.008
DXA fat mass	69	-0.08	±1.00	0.214	-0.295	0.014
DXA limb fat mass	69	-0.06	±0.98	0.324	-0.294	0.014
DXA trunk fat mass	69	-0.03	±1.02	0.619	-0.304	0.011
Bicep skinfold thickness	69	-0.17	±1.32	0.037	0.118	0.336
Tricep skinfold thickness	68	-0.06	±1.32	0.473	0.002	0.989
Subscapular skinfold thickness	69	-0.13	±1.38	0.122	0.214	0.077
Supra-iliac skinfold thickness	66	0.04	±1.20	0.583	0.032	0.801
Sum 4 skinfold thicknesses	65	-0.06	±1.04	0.356	-0.160	0.204
<i>Measures of 'leanness'</i>						
DXA fat-free mass	69	-0.02	±0.60	0.578	0.162	0.183
DXA limb fat-free mass	69	0.04	±0.68	0.308	0.007	0.953
DXA trunk fat-free mass	69	-0.04	±0.70	0.360	0.325	0.006
Total body water	69	0.04	±0.62	0.351	0.271	0.024
Bio-electrical impedance	32	0.19	±0.72	0.005	0.260	0.151
Corrected bio-electrical impedance ²	32	0.07	±0.68	0.283	0.027	0.884
Combined DXA and BIA	32	0.03	±0.34	0.283	-0.156	0.394

¹ Bias is the difference (simple technique – 4-component model (4CM)), 95% limits of agreement; ±2SD, DXA; dual-energy X-ray absorptiometry, BMI; body mass index, BIA; bio-electrical impedance. ²Corrected BIA SDS; BIA SDS – predicted bias calculated by regressing BIA SDS on bias BIA SDS adjusting for age and BMI SDS. P^{bias} ; independent t -test compared to zero, R ; Pearson's correlation, $P^{\text{correlation}}$; paired sample t -test between the bias and the mean of simple technique and 4CM.

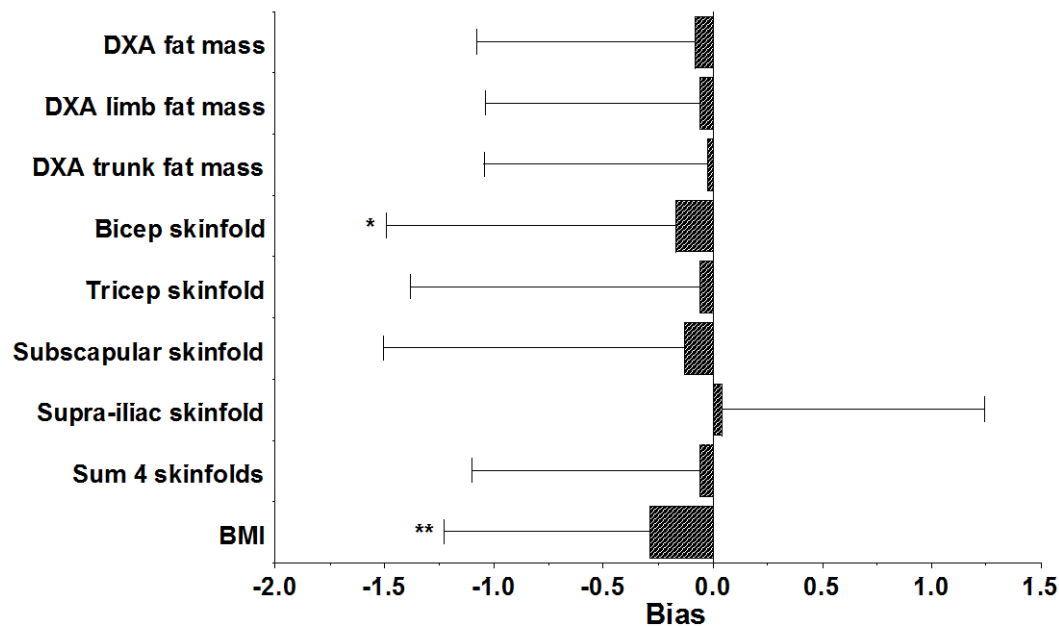


Figure 8.3. Bias and limits of agreement ($\pm 2SD$) for change in 'fatness' by simple techniques compared to the 4-component model (4CM; change by simple technique SDS – change by 4CM FM SDS). All values are SDS, DXA; dual-energy X-ray absorptiometry, BMI; body mass index. Asterisks denote biases that were significantly different from the 4CM, * $p < 0.05$, ** $p < 0.001$.

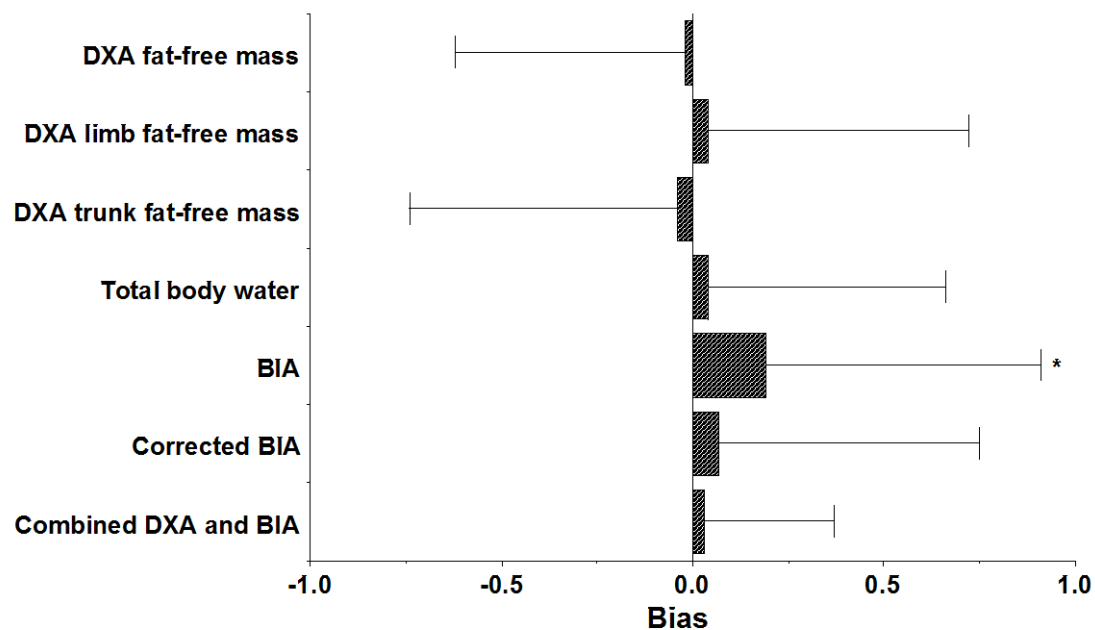


Figure 8.4 Bias and limits of agreement ($\pm 2SD$) for change in 'leanness' by simple techniques compared to the 4-component model (4CM; change by simple technique SDS – change by 4CM FFM SDS). All values are SDS, DXA; dual-energy X-ray absorptiometry, BIA; bio-electrical impedance. Asterisks denote biases that were significantly different from the 4CM, * $p < 0.005$.

8.6.5 Assessment of the ability of each technique to distinguish ‘normal’ and ‘abnormal’ body composition in clinical practice.

The ability of each simple technique to identify ‘normal’ and ‘abnormal’ body composition (normal defined as ± 2 SDS 4C FM or 4C FFM) was examined by % agreement and Cohen’s Kappa coefficient (**Table 8.6**). DXA showed the highest agreement (96.2%) and kappa value (0.73) with 4C FM SDS. Although agreement for SFT and BMI was high (91-94%) the kappa coefficient which takes account of chance agreement was much lower at 26-44%.

Agreement of simple techniques with 4C FFM indicated that TBW had the highest agreement (97 %) and kappa value 83%. Whole body DXA FFM and FFMI agreed with 4C FFM and FFMI SDS by approximately 96% with a kappa value of 76.5%. The ability of regional FFM to agree with 4C FFM was reduced with kappa values of 0.57 for limb and 0.71 for trunk and reduced much further when size adjusted; limb 0.33 and trunk 0.34. Corrected BIA SDS had slightly better agreement with 4C FFM than unadjusted BIA (72 v 69%) and combined DXA and corrected BIA SDS a similar agreement to DXA on its own.

Table 8.6. Cross-tabulation statistics for agreement in ‘abnormal’ scores assessed by simpler techniques compared to the 4-CM.

Measurement method (SDS)	N	% agreement	<i>k</i>	<i>se</i>	% abnormal by 4CM	% abnormal by other technique
<i>Measure of ‘fatness’</i>						
DXA fat mass	266	96.2	0.73	0.08	8.6	6.4
DXA fat mass index	266	96.6	0.69	0.10	6.8	4.9
DXA limb fat mass	266	95.8	0.72	0.08	8.6	7.5
DXA limb fat mass index	266	96.6	0.71	0.09	6.8	5.6
DXA trunk fat mass	266	94.8	0.64	0.09	8.6	7.1
DXA trunk fat mass index	266	95.8	0.65	0.10	6.8	5.6
Bicep skinfold	265	92.4	0.26	0.11	8.6	1.9
Tricep skinfold	265	91.3	0.37	0.10	8.7	6.0
Subscapular skinfold	266	92.8	0.39	0.11	8.6	3.8
Supra-iliac skinfold	257	93.8	0.44	0.11	8.2	3.5
Sum 4 skinfold	255	93.0	0.29	0.12	8.2	2.0
BMI	266	91.7	0.35	0.11	8.6	4.9
<i>Measure of ‘leanness’</i>						
DXA fat-free mass	266	94.8	0.76	0.06	11.3	14.3
DXA fat-free mass index	266	97.0	0.77	0.08	6.0	8.3
DXA limb fat-free mass	266	88.0	0.57	0.07	11.3	21.9
DXA limb fat-free mass index	266	86.8	0.33	0.08	5.7	15.9
DXA trunk fat-free mass	266	94.3	0.71	0.07	11.3	10.9
DXA trunk fat-free mass index	266	94.7	0.34	0.13	6.0	2.3
Total body water	266	96.6	0.83	0.05	11.3	11.7
Bio-electrical impedance	141	92.2	0.69	0.09	13.5	15.6
Corrected bio-electrical impedance	141	84.6	0.72	0.09	13.5	12.8
Combined DXA and BIA	141	93.5	0.74	0.08	13.5	15.7

DXA; dual energy X-ray absorptiometry, BIA; bio-electrical impedance, index is height²

8.6.6 Accuracy of an aggregate prediction using the ‘wisdom of crowds’ approach.

87 children (41 boys) had data that fitted the criteria for all 12 prediction equations (**Table 8.7**). Characteristics of the children are presented in **Table 8.8**, they were significantly shorter than the UK reference data ($p<0.05$) and the girls were significantly lighter ($p<0.01$). For the prediction of FM and FFM (since they are equal and opposite) the aggregate prediction had the third lowest bias (0.8 kg; $p<0.001$) and the second lowest limits of agreement (± 4.2 kg) (**Table 8.9, Figure 8.5**). The individual predictions range in bias from 0.5 to 7.2 kg and limits of agreement of ± 3.6 kg to ± 15.5 kg. For 8 of the prediction equations the magnitude of the bias was significantly related to the magnitude of FM, this was not the case for the aggregate prediction or 4 of the individual predictions.

Figure 8.6 shows the bias in FM for individual children for the aggregate equation. Six children had a bias greater than 4 kg FM which for 5 children was between 47 and 140% of their FM by 4CM.

Table 8.7. Equations for the prediction of body composition in children

Raw data	Predicted variable	Age range y	Equation	Groupings
Weight and height				
Morgenstern et al (232)	TBW	0.25-13	$0.0846 \times 0.95F \times (Ht \times Wt)^{0.65}$	
	TBW	>13	$0.0758 \times 0.84F \times (Ht \times Wt)^{0.69}$	
Mellits and Cheek (233)	TBW	0.1-31	$-1.927 + (0.465 \times Wt) + (0.045 \times Ht)$	Boys ≤ 132.7 cm
	TBW		$-21.933 + (0.406 \times Wt) + (0.209 \times Ht)$	Boys ≥ 132.7 cm
	TBW		$0.076 + (0.507 \times Wt) + (0.013 \times Ht)$	Girls ≤ 132.7 cm
	TBW		$-10.313 + (0.252 \times Wt) + (0.154 \times Ht)$	Girls ≥ 132.7 cm
BMI				
Deurenberg et al (234)	% Fat	7-20	$1.4 + (1.51 \times BMI) - (0.70 \times age) - (3.6 \times M)$	
Pietriobelli et al (235)	FM	5-19	$-29.91 + (2.06 \times BMI)$	Boys
	FM		$-30.65 + (1.90 \times BMI) + (0.53 \times age)$	Girls
Skinfold thickness				
Slaughter et al (165)	% Fat	8-29	$(1.21 \times SF2) - (0.008 \times SF2^2) - 1.7$	Boys SF2 <35 mm
	% Fat		$(1.33 \times SF2) - (0.013 \times SF2^2) - 2.5$	Girls SF2 <35 mm
	% Fat		$(0.783 \times SF2) - 1.7$	Boys SF2 >35 mm
	% Fat		$(0.546 \times SF2) + 9.7$	Girls SF2 >35 mm
Johnston et al (164)	BD	8-14	$1.279 - (0.121 \times \log SF4)$	Boys with CF
	BD		$1.120 - (0.030 \times \log SF4)$	Girls with CF
Deurenberg et al (236)	BD	Mean 11	$1.1133 - (0.0561 \times \log SF4) + (1.7 \times age \times 10^{-3})$	Boys
	BD	Mean 10.5	$1.1187 - (0.063 \times \log SF4) + (1.9 \times age \times 10^{-3})$	Girls

F; female = 1, male = 0, M; female = 0, male = 1, Wt; weight, Ht; height, TBW; total body water (L), BD; body density (g/cm³), SF2; sum of tricep and subscapular skinfold thicknesses (mm), log is log to base 10, SF4; sum of bicep, tricep, subscapular and suprailiac skinfold thicknesses (mm), FM; fat mass, FFM; fat-free mass. **Table continues on next page.**

Table 8.7 continued. Equations for the prediction of body composition in children

Raw data	Predicted variable	Age range y	Equation	Groupings
Ht²/Z				
Horlick et al (237)	TBW	4-18	$0.725 + (0.475 \times \text{Ht}^2/\text{Z}) + (0.14 \times \text{Wt})$	
Houtkooper et al (90)	FFM	10-19	$(0.61 \times \text{Ht}^2/\text{Z}) + (0.25 \times \text{Wt}) + 1.31$	
Schaefer et al (238)	FFM	3-19	$(0.65 \times \text{Ht}^2/\text{Z}) + (0.68 \times \text{age}) + 0.15$	
Deurenberg et al (91)	FFM	7-15	$(0.406 \times \text{Ht}^2/\text{Z}) + (0.36 \times \text{Wt}) + [5.58 \text{ Ht (m)}] + (0.56 \times \text{M}) - 6.48$	
Cordain et al (239)	FFM	9-14	$6.86 + (0.81 \times \text{Ht}^2/\text{Z})$	

F; female = 1, male = 0, M; female = 0, male = 1, Wt; weight, Ht; height, TBW; total body water (L), BD; body density (g/cm³), SF2; sum of tricep and subscapular skinfold thicknesses (mm), log is log to base 10, SF4; sum of bicep, tricep, subscapular and suprailiac skinfold thicknesses (mm), FM; fat mass, FFM; fat-free mass, Z; impedance (ohms).

Table 8.8. Characteristics of children whose data was used for the aggregate prediction

	Boys n=41				Girls n=46			
	Mean	SD	Range		Mean	SD	Range	
Age (y)	12.8	1.91	10.1	16.0	12.68	1.76	10.0	15.7
Weight (kg)	44.0	12.2	25.8	86.6	40.8	10.1	22.2	61.4
Height (m)	1.52	0.14	1.29	1.83	1.48	0.11	1.21	1.68
BMI (kg/height ²)	18.6	2.28	13.9	25.9	18.4	2.68	13.2	24.5
Weight SDS	-0.02	0.98	-1.89	2.07	-0.56**	1.31	-4.45	1.42
Height SDS	-0.39*	1.15	-2.53	1.97	-0.72***	1.29	-3.64	1.46
BMI SDS	0.18	0.87	-1.92	1.90	-0.21	1.14	-3.78	1.57
Bicep skinfold (mm)	6.6	3.3	3.2	21.2	8.6	3.4	3.9	18.9
Tricep skinfold (mm)	10.5	3.7	4.7	18.8	13.4	4.6	6.1	24.5
Subscapular skinfold (mm)	7.4	2.6	4.0	17.9	10.5	6.3	4.6	31.6
Supra-iliac skinfold (mm)	12.1	6.8	5.2	34.3	15.9	8.2	5.5	33.7
Resistance (ohms)	695	82	539	867	800	88	584	1065
Total body water (l)	26.5	7.0	16.0	40.4	22.6	4.65	14.2	31.2
4-component fat mass (kg)	8.33	4.68	3.21	28.7	10.3	4.90	3.18	21.9
4-component fat-free mass (kg)	35.7	9.83	21.8	57.9	30.5	6.35	19.0	43.0

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, one sample t -test compared to zero, child with CF compared to reference data.

Table 8.9. Bland-Altman statistics for bias and limits of agreement for individual or aggregate equations and reference 4-component fat mass.

Equation	Bias ¹	Limits of agreement	<i>P</i>	<i>R</i>	<i>P</i> ^{correlation}
Deurenberg et al, BIA	7.17	± 8.91	<0.001	0.372	<0.001
Johnston et al	-5.84	±15.47	<0.001	0.488	<0.001
Horlick et al	4.66	± 6.62	<0.001	0.233	0.030
Schaefer et al	4.19	± 6.44	<0.001	0.514	<0.001
Morgenstern et al	-3.14	± 8.98	<0.001	-0.140	0.196
Houtkooper et al	2.33	± 5.28	<0.001	0.013	0.908
Cordain et al	1.22	± 6.95	0.001	0.500	<0.001
Deurenberg et al, BMI	-1.22	± 5.00	<0.001	-0.599	<0.001
Deurenberg et al, SFT	0.91	± 7.11	0.002	0.049	0.654
Slaughter et al	-0.89	± 3.56	<0.001	-0.219	0.041
Aggregate	0.77	± 4.17	0.001	-0.168	0.120
Mellitis and Cheek	-0.60	± 5.61	0.049	-0.029	0.788
Pietrobelli et al	0.46	± 5.82	0.143	0.150	0.165

¹Difference (prediction equation – 4-component model (4CM)), bias in fat-free mass is equal and opposite. Limits of agreement; ±2SD, *P*; one-sample t-test of bias compared to zero, *R*; correlation between the mean value (4CM and individual prediction equation) and the bias.

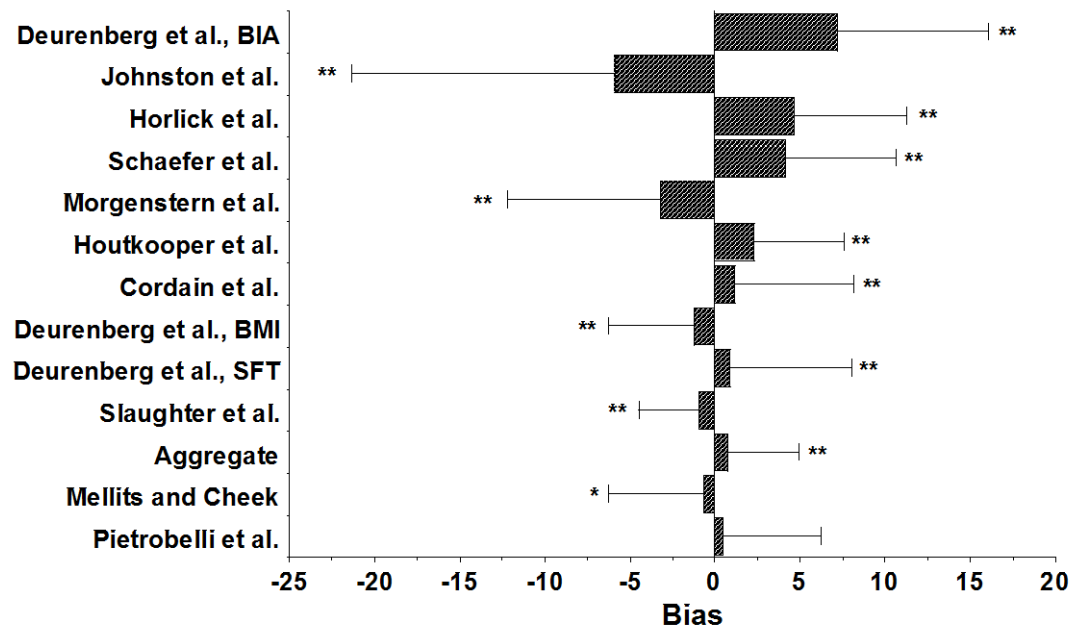


Figure 8.5. Bland-Altman analysis showing mean bias (predicted – 4CM) and limits of agreement ($\pm 2SD$) in fat mass and fat-free mass in 107 children with CF compared to the 4-component model. The bias in fat-free mass is equal and opposite to that of fat mass. The graph compares 12 individual predictions and an aggregate average of the 12 equations to the 4-component model. BIA; bio-electrical impedance, BMI; body mass index, SFT; skinfold thickness. Asterisks denote biases that were significantly different from the 4CM, * $p < 0.05$, ** $p < 0.005$.

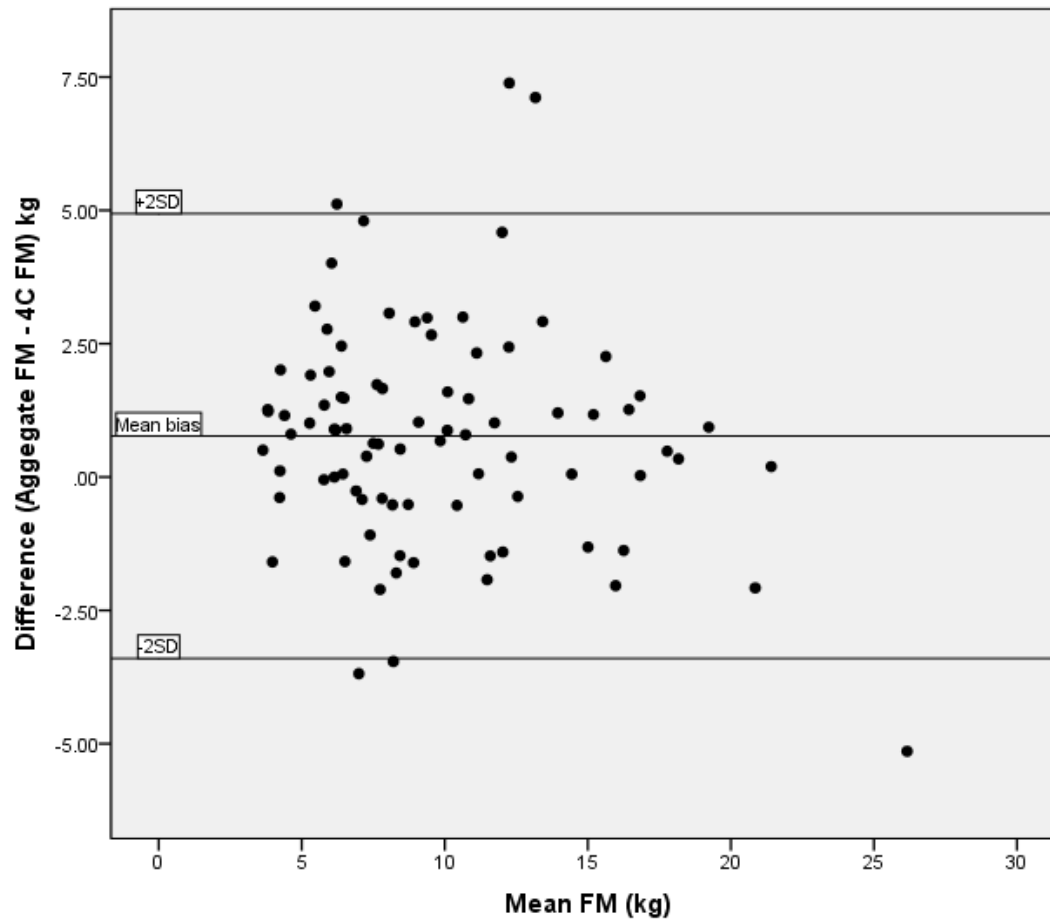


Figure 8.6 Individual bias and limits of agreement ($\pm 2SD$) for fat mass(FM) by the aggregate prediction compared to the 4-component model (4C FM;(aggregate FM – 4CM FM).

8.7 Discussion

The 4CM is the ‘criterion’ in vivo method for the assessment of body composition in children but is not widely available due to high cost, the need for skilled technicians and lack of portability of the equipment. Other simpler techniques, which are available in the hospital setting are therefore the methods of choice for clinical assessment. It is important that these techniques are assessed against a criterion method in order to identify the most appropriate for assessing body composition in children with CF and to determine to what extent they are interchangeable. This is important because not all techniques may be available at every assessment or suitable for all patients on all occasions.

DXA may be available in most hospitals but its use in children tends to be in specialist paediatric centres since interpretation of the data obtained is complicated by body size and maturity (213). Several studies in adults and children have assessed absolute values for FM and FFM against the 4CM and found relatively poor agreement in terms of mean bias and limits of agreement (102;167;198;199;226;240-244) with age, gender, size, pubertal status and disease state as possible factors influencing the bias. A recent study from our centre (245) found that when using DXA to assess body composition in obese children the bias was negligible but the limits of agreement wide for FM (± 3.2 kg) and FFM (± 3 kg). These findings suggest that the use of DXA for individual assessment and longitudinal change has limitations.

The use of body composition measurements in clinical practice is hampered by lack of reference data, particularly in children. Several datasets have been generated for techniques such as SFT, BIA and DXA (246-252) but none have generated reference data in the same population using several different techniques. This issue was recently addressed at our centre by measuring 533 children, adolescents and young adults to generate reference curves from which SDS can be calculated for 4C FM and FFM and many of the simpler methods (155). Using this data I have compared SDS for BMI, SFT

and DXA FM to 4C FM and BIA, TBW and DXA FFM to 4C FFM. These comparisons identify the best simple technique to use to assess FM and FFM in children with CF aged 6 -17 years.

It is conceivable that there may be occasions when only simple bedside techniques such as anthropometry, SFT and BIA are available due to lack of resources. Prediction of FM or FFM from a single prediction technique, particularly in children and patients, is likely to be inaccurate and imprecise. However, an aggregate prediction in a 'wisdom of crowds' approach has been shown to improve accuracy and precision (253) when assessing body composition in healthy children and adolescents.

There are 4 aspects to my analysis: (1) cross sectional comparison of simple techniques and the 4CM, (2) longitudinal comparison, (3) a comparison of the classification of 'abnormal' scores by simple techniques and the 4CM and (4) whether an aggregate prediction is more accurate and precise than individual predictions. These analyses aim to identify which techniques can be used to measure CF children's body composition and monitor change adequately and the extent to which they may be interchangeable. The ability of a technique to classify patients as 'normal' or 'abnormal' for FM and FFM is an important factor when designing treatment and dietary regimens and therefore these analyses aim to quantify this for all simple techniques. Finally, it must be acknowledged that many of the options may not be available for all patients at all times and therefore I have included an assessment of a method using the simplest bedside techniques.

8.7.1 Cross sectional comparison of simple techniques compared to the 4-component model and classification of patients

I found DXA to be a more accurate predictor of 4C FM than BMI or SFT. Bias in DXA whole body and regional FM was small (<0.16 SDS) and limits of agreement ranged from ± 0.4 (trunk) to ± 0.7 (limbs). The best predictor of 4C FFM was TBW which is

unlikely to be used clinically due to its high cost and lengthy analysis time. DXA whole body and trunk FFM bias was small (<0.2 SDS) and DXA limb FFM underestimated by on average, 0.5 SDS. Limits of agreement ranged from 0.4 to 0.8. Although whole body FM and FFM did not show the lowest limits of agreement compared to regional DXA, it would be wise to use whole body scans in clinical practice because the accuracy of regional scans will be affected by the positioning of the 'regions of interest' by the operator. Whereas the region of interest is chosen by the operator for regional scans in whole body scans it is standardised. In addition, the bias in whole body FM and FFM were not affected by age, sex or BMI SDS in this group of children.

For assessing FFM, corrected BIA showed no bias but wider limits of agreement than DXA whole body. However, a combination of DXA whole body FFM and BIA SDS gave no bias and narrower limits of agreement (0.53 SDS) than most of the other single techniques. Since BIA is a cheap, easy technique which could be performed in the same room as the DXA it would seem prudent to combine both techniques for the assessment of FFM. A database to calculate the corrected BIA and combined SDS could be provided in Excel format although the correction factor would need to be tested in other populations before being applied to other groups children with CF of the age range in this study.

Whole body DXA showed good agreement with 4CM for categorising patients as 'normal' and abnormal' with regards to FM and FFM (73% and 76% respectively) and DXA FFM combined with BIA SDS was similar. The use of DXA (with or without BIA) in clinical practice as a predictor of 4C FM and FFM SDS and in the identification of CF children with abnormal body composition is supported by these findings. However, it is important to note that the limits of agreement were wide with 95% of values from DXA whole body falling within a 1.5 or 1.3 SD band of those obtained by 4C FM and FFM respectively. This suggests that DXA cannot be considered to be interchangeable with the 4CM and that the results should be used in conjunction with other clinical indicators. If BIA is used in combination with whole body DXA FFM, 95% of values fall within a narrower band of 0.5 SDS. DXA was also less accurate in

patients with low FM. The finding that the bias and magnitude were correlated for both whole body and regional FM and whole body FFM is in accord with previous research demonstrating that size affects the measurement of soft tissue from DXA (102;196;254).

Analysis using indexed (height adjusted) DXA variables compared to 4C FMI and FFMI did not improve the results although there was an unexpected finding for trunk FFMI. In both boys and girl it appears that there is a depletion of FFM by 4CM and DXA whole body (Table 6.1) that is not replicated in the trunk if a height adjustment is made. On the contrary, there is a positive mean value of 0.65 for boys and 0.28 for girls. One possible explanation is that although these CF children are short for their age the shortness may be disproportionate in the limbs and trunk and therefore height² would not be the most appropriate power for both trunk and limbs. Data on sitting height is not available for baseline and some of year 2 measurements although 127 subsequent measurements of this group of children indicate a mean (SD) for standing height SDS of -0.57 (1.07) and sitting height SDS of -0.67 (1.11), $p=0.452$. Another explanation is that this finding is a reflection of increased density in the trunk area which may be due to enlarged organs or may be as a result of bowel dysfunction or methodological issues with DXA instrumentation as discussed in Chapter 6. Height adjusting regional FFM by DXA reduces agreement on classification of patients by about a half compared to unadjusted and therefore the use of trunk FFM SDS for clinical assessment cannot be recommended.

DXA may not always be available in clinical practice and therefore the ability of BMI and SFT to predict 4C FM and BIA to predict 4C FFM is an important consideration. BMI and SFT provided much less agreement with 4C FM than DXA whole body FM, demonstrating large positive biases and wide limits of agreement. Tricep SFT provided a slightly better ranking of 4C FM than the other 3 SFT or BMI although the limits of agreement were similar. Agreement with 4CM to categorise patients as 'abnormal' was also poor. It is therefore not advisable to use SFT or BMI as an indicator of FM, even as a crude method to monitor an individual child with CF longitudinally. However, corrected BIA showed no bias (and good agreement for classification of patients) and

therefore when DXA is not available BIA, which is a cheap readily available technology, can be used to assess FFM, although it should be noted that limits of agreement are wide at ± 0.8 SDS. As previously discussed combining DXA and corrected BIA SDS improved accuracy and precision compared to DXA on its own. However, this data is obtained from a single frequency, standing BIA machine and caution should be taken if using a different make, model, multi-frequency or supine measurement machine.

All techniques demonstrated an over-estimation of FM compared to 4C FM whereas bias in FFM estimation was variable.

8.7.2 Longitudinal comparison of simple techniques compared to the 4-component model

Change in DXA whole body and regional FM and FFM SDS did not show any significant bias compared to change in the 4CM SDS although bias in DXA FM (whole body and regional) was greater in those with the least change and bias in change of DXA trunk FFM greatest in those with the most change. Limits of agreement were about 1 SDS for change in FM and slightly smaller for FFM. The use of DXA whole body scans is recommended for clinical practice to reduce operator error. Combining DXA FFM and BIA SDS not only improves the cross sectional outcome but also reduces the limits of agreement for longitudinal change by half. The use of DXA whole body FM and FFM with or without corrected BIA in clinical practice to measure body composition and monitor change is supported by these findings. However, it is important to note that the limits of agreement for bias in change in DXA whole body FM and FFM are wide with 95% of values from DXA falling within a 2.0 or 1.2 SD band of those obtained by 4C FM and FFM respectively. For FFM this is reduced by combining BIA with DXA whole body FFM (0.7 SD). Corrected BIA SDS had an insignificant bias compared to change in 4C FFM with limits of agreement close to those of DXA and therefore would be a good alternative to assess FFM when DXA is not available. The limits of

agreement suggest that these techniques should not be used interchangeably however; using the same technique over time will best monitor change in body composition.

8.7.3 ‘Wisdom of crowds’ aggregate prediction from several simple prediction techniques

As hypothesised by the ‘wisdom of crowds’ theory the aggregate prediction had greater accuracy than the majority of individual prediction equations in estimating FM and FFM in this group of children with CF. Accuracy was better than all but 2 individual prediction equations and precision better than all but 1 individual prediction equation. Most of the individual prediction equations produced values where the magnitude of the bias was related to the magnitude of FM or FFM. This was not the case for the aggregate prediction. Although mean bias in the aggregate prediction compared to 4C FM and FFM was one of the lowest (0.77 kg), individual bias was as much as 4 kg which, for 6 children was between 50 and 139% of their 4C FM or FFM. Clearly, where better techniques are available these should be used, but where they are not feasible this approach has some merit for assessing groups.

The ‘wisdom of crowds’ approach minimises the risk of selecting an equation with a high bias given that it is not possible to know which equation will perform best in any given group. Although 2 equations were marginally better than the aggregate for agreeing with the 4CM this may not be the case in another population of children with CF. In addition the aggregate prediction had narrower limits of agreement than 11 of the 12 individual prediction equations. It should be noted that all but 1 of the equations used were generated in healthy populations and therefore further work to generate more CF specific equations, using several bedside techniques, would be helpful to determine whether accuracy and precision may be improved.

8.8 Study limitations

There are some limitations to this study. Firstly, it should be considered whether the fact that DXA is compared on its own to the 4CM which utilises DXA BMC measurement may influence the result. I have previously addressed this issue (102) by analysing data from both 4CM and the 3CM which does not utilise any DXA measures. The results remained unchanged. Secondly, no attempt has been made to relate these findings to clinical outcome so although, for example, SFT are not good at assessing absolute FM they may be useful clinically to monitor health. Thirdly, these analyses have utilised standardised scores, and the question of whether there is bias in absolute FM and FFM kg has not been addressed. Fourthly, I used standing BIA apparatus and the findings may be different where impedance is measured lying down. Finally, prediction equations generated with data from children with CF rather than healthy children may improve the outcome of an aggregate prediction.

8.9 Summary

- Techniques cannot be used interchangeably.
- Standardised FM and FFM measurements from DXA compare most favourably with those derived from the 4CM and are the most useful clinically for measuring body composition and monitoring change.
- Regional DXA may be affected by operator error whereas whole body DXA is standardized and therefore whole body scans are recommended for clinical practice.
- BIA may reasonably be used to provide a FFM SDS in place of DXA if a correction factor is applied. Assessment of FFM is improved by combining corrected BIA with DXA FFM in terms of agreement with the 4CM in this group of children.
- Despite the short stature of these CF children, agreement with 4CM is not improved by height adjusting DXA SDS.
- BMI and SFT should not be used for assessing individuals due to lack of accuracy.
- Monitoring change in body composition is appropriate with DXA, BIA or a combination of the 2, although the separate techniques are not interchangeable over time.
- When only simple bedside techniques are available the use of an aggregate prediction based on a healthy population may be informative when comparing groups but not individuals. It is likely that the generation of several prediction equations based on data from children with CF and aggregated will improve the outcome.
- Ultimately, the most appropriate measure should be determined by its predictive value for clinical outcome.

Chapter 9. Conclusion

This chapter will summarise the findings, describe possible future research, outline the limitations of the thesis and relate the findings to clinical practice.

9.1 Summary of the findings

This thesis had 3 aims;

4. To investigate the effect of CF on body composition in young children with CF and whether this changes with growth and maturity. In addition, to investigate whether the type of comparison (pair-, group-match or compared to a reference population) affects the outcome of the analysis.
5. To investigate the relationship between body composition and clinical status assessed by FEV₁.
6. To investigate simple body composition techniques to define which would be most appropriate in clinical practice where 4CM is not available.

9.1.1 Effect of cystic fibrosis on body composition

Even at baseline (Chapters 5 and 6), abnormal body composition was apparent with clear sex differences indicating that abnormalities in females may be established much earlier than previously considered. The deficit of FM and MM found in this group of girls with CF was not related to pubertal status. Two girls had hidden depletion of FFM which is likely to impact greatly on the course of the disease and response to treatment. Both girls and boys with CF were short but boys had similar body composition to control boys apart from more FFM once adjustment for stature was made. However, 3 boys were obese which may present problems for the future management of these patients. Only by performing body composition measurements was it possible to identify those boys with high BMI and high FM or FFM and those girls with 'normal' BMI and low FFM indicating that, without these measurements a

detrimental body composition may not be identified clinically. The consistent finding was that the children with CF have, on average, larger waist circumference than both the reference data and controls which appears from regional DXA measurements to be a reflection of increased FFM rather than increased FM, suggesting that waist measurements will not be helpful as a measure of adiposity in this group.

Longitudinal analysis (Chapter 7) indicated deterioration in FFM in both sexes although there was an indication of improving FFM in the older girls. Boys lowest in FFM at baseline and girls lowest in MM at baseline showed the greatest 'catch-up' (or least decline) in these tissues over 2 years. Clinically, BMD SDS is monitored and in children this is size adjusted as BMAD SDS which, for the children in this study remained stable at 0.2 in boys and -0.4 in girls. I also investigated the role of physical activity on body composition and although there was no relationship between baseline activity and body composition at 2 years, the most active boys at 2 years had less than expected gains in FM and FFM gains similar to controls. However, it must be emphasised the assessment of activity in this study was crude and a causal relationship between activity and body composition could only be defined with an intervention study.

None of these effects would have been detected using simple anthropometric measurements.

Different types of analysis were used to compare the children with CF to a healthy control group; matched-pair and group comparisons were made with absolute values of components of body composition and also SDS calculated from a large reference population. In addition, these SDS indicate how both the children with CF and the controls compare to the large reference population. I found that the conclusions differed depending on the type of analysis and this may, in part, explain the differing conclusions of previous studies.

9.1.2 The relationship between body composition and spirometry as a marker for clinical status.

I found that FM in girls was related to FEV₁ at baseline but not at 2 years despite declining lung function. No relationship between body composition and spirometry was found in boys at baseline although at 2 years FFM was related to FEV₁ and in girls whole body BMC was related to FEV₁ in accord with many previous studies. These findings are presented in Chapters 5 and 6. In Chapter 7 I showed how a greater than expected increase in FM in boys was associated with greater increase in FEV₁ over 2 years and that baseline FEV₁ in boys predicted change in FFMI SDS such that a 1 SDS higher FEV₁ at baseline predicted an additional 0.18 SDS change in FFMI over 2 years. These findings suggest that, in boys, improving lung function is associated with greater than expected gains in FM over the same period, and that baseline lung function predicts greater than expected subsequent gain in FFM. However, the relationship between body composition and lung function is clearly complex and it is impossible to define cause and effect with an observational study. A randomised controlled intervention trial to improve either body composition or lung function would be necessary.

This study indicated that BMI SDS was only related to FEV₁ when there was a severe depletion of FM in girls at baseline but it did not relate to change in FEV₁ over the 2 years and in addition, does not accurately reflect either obesity or depletion of FFM. This highlights the importance of using body composition measurements in clinical practice if the patient is to receive the most appropriate treatment.

9.1.3 Simple body composition techniques for use in clinical practice where 4-component model is not available

When deciding which body composition technique to use in clinical practice there are practical considerations such as cost, availability, ease of use and patient acceptance and also consideration of the quality of the data derived from the technique and the applicability to the question being asked. For example, SFT are

not appropriate to measure FM in the group of children measured for this thesis but SFT may be useful to monitor overall health in these children. In addition, a technique which has wide limits of agreement may not be suitable to assess and monitor individuals over time but may be useful in large epidemiological studies.

I found that FM and FFM SDS derived from whole body DXA gave results closest to the criterion method and that assessment of FFM is improved by combining adjusted BIA with DXA in this group of children. However, the equation generated to calculate adjusted BIA values in this study would need to be tested in other similar aged CF populations before its use could be recommended generally. BMI and SFT are not accurate enough to assess individuals. Monitoring change in body composition is best achieved with DXA, BIA or a combination of both although separate techniques are not interchangeable over time. Simple bedside techniques may be informative when comparing groups if an aggregate prediction (of several predictions) is used.

9.2 Future Research

From the work in this thesis I have identified possible areas of future research:

1. In the light of the findings of altered body composition in this group of children there is a need to establish how body composition measurements can be used clinically, for example:
 - a. Continued longitudinal follow-up of this cohort into adult life may identify whether baseline or change in body composition predicts later prognosis.
 - b. Could body composition measurements be used as a basis for nutritional advice, for example, is it better to base calculation of energy requirement on FFM rather than weight? A randomised controlled trial of current (based on weight) versus new management (based on FFM) may be helpful.
 - c. Whether nutritional interventions lead to greater gains in FM or FFM and whether gains in FM or gains in FFM have different outcomes.

- d. Randomised controlled trials of the impact of interventions on lung function or body composition to better understand the relationship between the two.
 - e. Using data from the 4CM to investigate the relationship between PM and lung infection.
2. Continued longitudinal follow-up of the cohort will also allow for the investigation of sex differences, not only in body composition but also activity using activity monitors and measures of behaviour and attitude to illuminate whether there are other, non-biological factors that contribute to sex differences. This information could then be used to design sex specific management.
3. Study of the generalisability of the equation generated to adjust raw values of impedance from BIA by comparing with deuterium dilution in another group of CF patients aged 6-17 years.
4. This thesis highlights some of the issues related to different measurement techniques and study design which may explain inconsistencies of previous research. Greater attention to these issues in future studies with more consistency in methods and study design would be an advantage.

9.3 Limitations

Methodological limitations have been discussed in each chapter and are summarised here;

- Spirometry is commonly used as a clinical outcome measure but may not capture a complete picture of the health of the patient. Ideally other clinical factors would be incorporated into the analysis although obtaining accurate records of all possible variables is difficult in this group of children where

some are managed at a local hospital and attend Great Ormond Street Hospital for Children once a year.

- I did not measure FEV₁ in the control children, however the reference data for lung function used in this study is from a large reference population and therefore should accurately depict the lung health of CF children in this study. However, it must be acknowledged that spirometry outcome is affected by motivation, particularly in the young.
- Consistent body volume measurements using ADP are more difficult to achieve due to erratic breathing in some of the children with CF. I therefore made multiple measurements in order to achieve consistency. Measured lung volumes whilst in the equipment are difficult to perform particularly in children and more so in children with CF. I therefore used predicted lung volumes in all the children. However, a previous study of children with CF using ADP found that there was no significant difference in FFM calculated using measured as opposed to predicted lung volume.
- The use of questionnaires to assess activity could be challenged since they may be affected by differing parental expectations between parents of children with CF and parents of healthy children.
- Children with CF have large changes in appetite and activity depending on how well they are. Measurement of body composition gives a 'snap shot' of that period and it is possible that there are changes occurring within the 2 year period that have been missed. However, treatment regimens for patients with CF are onerous and many take part in several research projects and therefore to increase the frequency of measurements would be unacceptable.
- Although I have found statistically significant differences between children with CF and controls and between different techniques this does not necessarily equate to clinical relevance. The -2 SDS cut-off for 'abnormal' is arbitrary and should only be regarded as a guide to be used in conjunction

with other clinical factors. Regarding techniques, the most appropriate measure should ideally be determined by its predictive value for clinical outcome; SFT may not be good to monitor FM but may be useful clinically to monitor health.

- Observational studies such as mine cannot define cause and effect. Only a randomised controlled trial of an intervention to improve body composition or improve lung function would make the picture clearer. However, due to the multiple factors which impact on overall disease severity in this group and the way that each is likely to impact upon and be affected by other factors, a clear answer is unlikely.
- SDS used in this study to compare techniques are, as yet, not generally available and will not be appropriate for all ethnic groups, all makes of DXA and supine BIA machines.
- This study was unable to investigate the important contribution of diet on body composition.

9.4 Implications of this research for clinical practice

The body composition of children with CF is an important factor influencing prognosis. Simple anthropometric techniques are internationally recommended to monitor the growth and nutritional status in these children. However, my findings suggest that these are not able to detect abnormal body composition. Using more detailed measurement techniques, abnormal body composition is apparent during mid-childhood, with gender differences that are in accord with our knowledge about prognosis. However, these findings would not be apparent unless an appropriate method is used, for example using simple anthropometry does not identify hidden depletion of FFM and 'obesity' and BMI is not a good predictor of FEV₁ in this group of children.

A very important aspect of this study is the information on which techniques are likely to be most appropriate for clinical use finding that DXA or a combination of DXA and BIA give results closest to the criterion method. However, the equation for adjusting BIA SDS would need to be tested in another population of children with CF before it could be recommended for other groups of CF patients.

The findings in this thesis support the concept that the measurement of body composition in children with CF is important for both research and clinical practice however, it must be emphasised that appropriate techniques should be used.

Acknowledgements

I cannot fully express my gratitude to my primary supervisor Dr Mary Fewtrell for her constant support and guidance and endless patience. The guidance of my secondary supervisors Dr Ranjan Suri and Professor Adam Jaffe has been invaluable and I am indebted to Professor Jonathan Wells who has given unstintingly of his time and expert knowledge. I would also like to express my gratitude to the many members of the respiratory medicine team at Great Ormond Street Hospital for Sick Children who have helped with recruitment and acquisition of clinical details, and those who performed lung function assessment and to my colleagues at the Childhood Nutrition Research Centre for their ongoing help, support and friendship.

I would like to express my appreciation to all the children who volunteered to take part in this research, some of whom I have watched grow and develop into adolescence and sadly a few have not. To their parents I thank them for giving time and encouragement to complete the measurements.

I am indebted to my loved ones for their encouragement and support during the entire PhD process and for never once saying, why?

Information about the work in this thesis

The work in this thesis was carried out at the Childhood Nutrition Centre, Institute of Child Health, University College London. The measurements were carried out in the Radiology Department, Great Ormond Street Hospital, London.

The project was conceived and developed with my supervisors and Professor Jonathan Wells. The children were recruited by Dr Christian Benden who assisted with collecting clinical details. I measured the children with the help of Catherine Wilson and the older children with CF, whose data was used in the analysis in Chapter 8, were measured using the same equipment by Dr Sirinuch Chomtho and myself. I processed the saliva samples, modeled the 4CM data and performed the analysis with expert advice from my supervisors, Professor Wells and with statistical input from Professor Tim Cole.

The body composition reference data was collected by myself and my colleagues; Catherine Wilson, Dalia Haroun, Sirinuch Chomtho and Kathy Kennedy using the same equipment used for this thesis. I modeled the data and contributed to the writing of two published papers related to the reference children.

Papers related to this thesis

I am the first author on a paper related to the baseline analysis of this group of children which is referenced as 69 in the bibliography and may be found in full at the end of the bibliography;

Williams JE, Wells JCK, Benden C, Jaffe A, Suri R, Wilson CM, & Fewtrell MS. Body composition assessed by the 4-component model and association with lung function in 6-12-y-old children with cystic fibrosis. *Am J Clin Nutr* 2010;92:1-12

I am co-author on two papers related to the reference data which are referenced as 184 (2010) and 155 (2012) in the bibliography and may be found in full at the end of the bibliography;

Wells JC, Williams JE, Chomtho S, Darch T, Grijalva-Eternod C, Kennedy K, Haroun D, Wilson C, Cole TJ, Fewtrell MS. Pediatric reference data for lean tissue properties: density and hydration from age 5 to 20 y. *Am J Clin Nutr* 2010; 91: 610-8

Wells JC, Williams J E, Chomtho S, Darch T, Grijalva-Eternod C, Kennedy K, Haroun D, Wilson C, Cole T and Fewtrell MS. Body composition reference data for simple and reference techniques and the four-component model: a new UK reference child. *Am J Clin Nutr* 2012 in press

I am first author on a methodological paper related to the techniques used in this thesis which is referenced as number 96 in the bibliography and may be found in full at the end of the bibliography;

Williams, J.E., Wells, J.C.K., Wilson, C.M., Haroun, D., Lucas, A. and Fewtrell, M.S. Evaluation of Lunar Prodigy dual-energy X-ray absorptiometry for assessing body composition in healthy persons and patients by comparison with the criterion four-component model. *Am J Clin Nutr.* 2006;83:1047-54.

Appendices

Appendix 1. Ethical approval

Institute of Child Health/Great Ormond Street Hospital Ethics Committee

The Institute of Child Health
30 Guilford Street
London
WC1N 1EH

Telephone: 020 7599 4130
Facsimile:

16 September 2008

Dr **M** S Fewtrell
Reader in Childhood Nutrition, Hon Consultant
Paediatrician Childhood Nutrition Research Centre,
UCL Institute of Child Health 30, Guilford St
London
WC1N 1EH
Dear Dr Fewtrell

Full title of study: Body composition and bone health in children with cystic fibrosis compared to healthy children; a longitudinal matched pairs study.

REC reference number: 08/H0713/66

Thank you for your letter of 01 August 2008, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair. **Confirmation of ethical opinion**

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). The favourable opinion for the study applies to all sites involved in the research. There is no requirement for other Local Research Ethics Committees to be informed or SSA to be carried out at each site.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>.

This Research Ethics Committee is an advisory committee to London Strategic Health Authority
*The National Research Ethics Service (NRES) represents the NRES Directorate within
the National Patient Safety Agency and Research Ethics Committees in England*

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application	1	05 June 2008
Investigator CV	1	04 February 2008
Protocol	1	01 May 2008
Covering Letter	1	27 June 2008
Compensation Arrangements	1	06 June 2008
Letter of invitation to participant	1	26 June 2008
Participant Information Sheet: CF parent information sheet	2	26 July 2008
Participant Information Sheet: Control over 12 yrs participant information sheet	2	26 July 2008
Participant Information Sheet: CF over 12 yrs participant information sheet	2	26 July 2008
Participant Information Sheet: Control child information sheet	2	26 July 2008
Participant Information Sheet: CF child information sheet	2	26 July 2008
Participant Information Sheet: Control parent information sheet	2	26 July 2008
Participant Consent Form: Assent	1	02 May 2008
Participant Consent Form: Parent	1	02 May 2008
Participant Consent Form: Participant	1	02 May 2008
Response to Request for Further Information	1	01 August 2008
CV Jane Williams		
Original Ethics Approval Letter	1	26 March 2008
Certificate for Participants	1	
Data Protection Form	1	

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review — guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencedroup_nres.npsa.nhs.uk.

081H0713/66 Please quote this number on all correspondence

With the Committee's best wishes for the success of

this project Yours sincerely



Dr Victor Larcher
Chair

[Email: Tom.Lucas@ich.ucl.ac.uk](mailto:Tom.Lucas@ich.ucl.ac.uk)

Copy to:
R&D office for GOSH/1CH

Dr Tracey Assari

Appendix 2.1. Child with CF information sheet

Great Ormond Street 
Hospital for Children
NHS Trust



MRC Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel 020 7905 2389

Title of project

Body composition and bone mineralisation in children with Cystic Fibrosis compared to healthy children.

Children's Information Sheet (6 to 11 yrs).

You are being asked to take part in a research study. Take time to decide if you want to say YES or NO to this. Please read this information, or ask someone to read it to you. Don't worry if you don't understand it straight away. Your parents have also been told about this, and you can ask them to help you understand. You can find more general information about research on the Great Ormond Street Hospital website:

(http://www.gosh.nhs.uk/gosh_families/research)

Why have I been asked to take part?

You have cystic fibrosis which means that you are not able to use all the food you eat to grow. We would like to measure you to find out how well you are growing.

Do I have to take part?

No, it is up to you to decide whether or not you want to take part. Even if you decide to take part, you can change your mind at any time and you don't have to give us a reason. If you decide not to take part it will not make any difference to your treatment.

What do I have to do?

You will be asked to come to the X-ray department at GOSH with your parents. It will take about 1 ½ hours and we will arrange a time when you are coming to the hospital for a routine appointment or are staying on the ward. None of the measurements are dangerous or hurt:

We will measure your size and shape with a tape measure.

We will ask you to lie on a bed with a camera over the top which takes a picture of your bones. You need to keep still for a few minutes while it takes a picture. You will get a copy of the picture of your skeleton to take home.

We will also take a picture of your arm and leg using another machine.

We will measure the strength of your arm and leg using sound waves and some slippery gel.

We will give you some cotton wool to put in your mouth to get wet and then give you a drink of special water. This will help us to measure how much water you have in your body.

We will ask you to sit in an egg shaped pod for a few minutes whilst wearing your swimming costume. There is no water, just air blowing gently around you.

We will ask you to stand on a weighing scale whilst you hold onto two handles.

We will ask you some questions about how healthy you are and what you eat.

We will ask you to wear an activity monitor on a belt around your waist so that we can measure how much you move about.

Photos of the machines are shown at the end of this leaflet. You will need to wear loose clothes without any metal – but you will not have to undress. Your parent or guardian will be with you all the time.

Is there anything dangerous?

None of the measurements are dangerous and none of them hurt. If you don't want to do one of the tests you can tell us.

Who will know about me taking part?

Only the people doing the research and your doctors at the hospital will know about you doing the measurements.

Who do I speak to if I have any problems?

You can speak to your parents who also have information about the study. You can also contact one of the researchers- their details are at the end of this leaflet. No one will be told that you have called and you do not have to give your name if you want to ask a question or talk about the tests.

Details of how to contact the researchers:

Jane Williams
Research Nurse
Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2389
Email; jane.williams@ich.ucl.ac.uk

Dr Mary Fewtrell
Chief Investigator
Childhood Nutrition Research
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2743
Email; m.fewtrell@ich.ucl.ac.uk

Thank-you for reading this information sheet!

a) Measuring your head



b) Taking a picture of your bones



b) You will be given a copy to keep



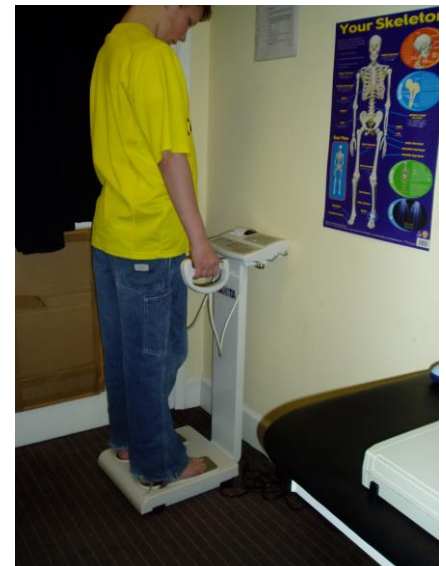
c) Taking another picture of your bones



d) How strong are your bones?



f) The Bodpod to measure your size and weight



g) Measuring your muscles

Appendix 2.2. Control child information sheet

Great Ormond Street 
Hospital for Children
NHS Trust



MRC Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel 020 7905 2389

Title of project

Body composition and bone mineralisation in children with Cystic Fibrosis compared to healthy children.

Children's Information Sheet (6 to 11 yrs).

You are being invited to take part in a research study. Take time to decide if you want to say YES or NO to this. Please read this information, or ask someone to read it to you. Don't worry if you don't understand it straight away. Your parents have also been told about this, and you can ask them to help you understand. You can find more general information about research on the Great Ormond Street Hospital website:

(http://www.gosh.nhs.uk/gosh_families/research)

Why have I been asked to take part?

We are measuring children with cystic fibrosis, an illness which means they are not able to use all the food they eat to grow. To find out how well they are growing we need to measure children who do not have any illness so we can see the difference. We are asking you to take part because you are healthy.

Do I have to take part?

No, it is up to you to decide whether or not you want to take part. Even if you decide to take part, you can still leave the study at any time and you don't have to give us a reason.

What do I have to do?

You will be asked to come to the X-ray department at GOSH with your parents. It will take about 1 ½ hours and we will arrange a time that is best for you and your parents. None of the measurements are dangerous or hurt:

We will measure your size and shape using a tape measure.

We will ask you to lie on a bed with a camera over the top which takes a picture of your bones. You need to keep still for a few minutes while it takes a picture. You will get a copy of the picture of your skeleton to take home.

We will also take a picture of your arm and leg using another machine.

We will measure the strength of your arm and leg using sound waves and slippery gel.

We will give you some cotton wool to put in your mouth to get wet and then give you a drink of special water. This will help us to measure how much water you have in your body.

We will ask you to sit in an egg shaped pod for a few minutes whilst wearing your swimming costume. There is no water, just air blowing gently around you.

We will ask you to stand on a weighing scale whilst you hold onto two handles.

We will ask you some questions about how healthy you are and what you eat.

We will ask you to wear an activity monitor on a belt around your waist so that we can measure how much you move about.

Photos of the machines are shown at the end of this leaflet. You will need to wear loose clothes without any metal – but you will not have to undress. Your parent or guardian will be with you all the time.

Is there anything dangerous?

None of the measurements are dangerous and none of them hurt. If you don't want to do one of the tests you can tell us.

Who will know about me taking part?

Only the people doing the research will know about you doing the measurements.

Who do I speak to if I have any problems?

You can speak to your parents who also have information about the study.

You can also contact one of the researchers- their details are at the end of this leaflet. No one will be told that you have called and you do not have to give your name if you want to ask a question or talk about the tests.

Details of how to contact the researchers:

Jane Williams
Research Nurse
Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2389
Email; jane.williams@ich.ucl.ac.uk

Dr Mary Fewtrell
Chief Investigator
Childhood Nutrition Research
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2743
Email; m.fewtrell@ich.ucl.ac.uk

Thank-you for reading this information sheet!

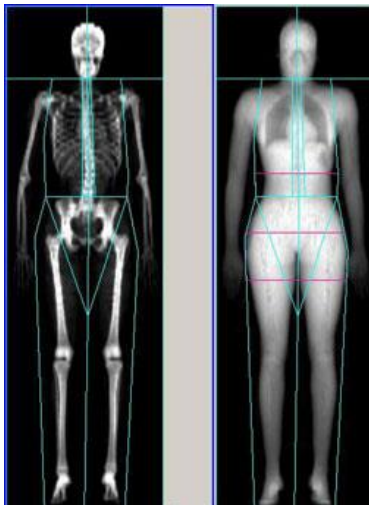
a) Measuring your head



b) Taking a picture of your bones



b) You will be given a copy to keep



c) Taking another picture of your bones



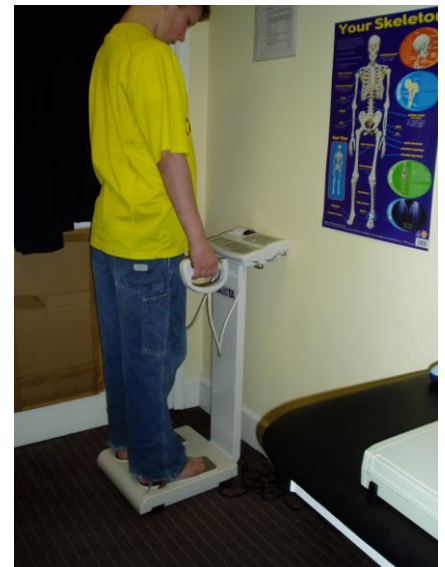
d) How strong are your bones?



f) The Bodpod to measure your size and weight



g) Measuring your muscles



Appendix 2.3. Child with CF over 12 y information sheet

Great Ormond Street 
Hospital for Children
NHS Trust



MRC Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel 020 7905 2389

Title of project

Body composition and bone mineralisation in children and with Cystic Fibrosis compared to healthy children.

Participant Information Sheet (12 yrs and older).

You are being invited to take part in a research study. Before you decide whether to take part or not, it is important you understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. You can find more general information about research on the Great Ormond Street Hospital website:

(http://www.gosh.nhs.uk/gosh_families/research)

What is the aim of the study?

The aim of this study is to measure the amount of fat, muscle and bone in children with cystic fibrosis (CF) and to see if these measurements relate to how well the children are.

Why is the study being done?

Some children with CF may develop problems with growth, due to problems with the absorption of food. This may lead to differences in the proportions of fat and muscle in the body, and also with the amount of mineral in their bones. Until recently, it has been difficult to accurately measure the amounts of fat, muscle and bone in children, but we can now do this using some simple measurements that are available at GOSH.

How is the study to be done?

We are inviting all children aged 6-17 years with CF who attend GOSH to participate. If you are willing to take part in the study, we will arrange an appointment at a time which is convenient for you (if possible, at the same time as your outpatient appointment). If you agree we will contact you again every 2 years to repeat the measurements. You can decide on each occasion whether to take part. The study will take about 1 ½ hours and will take place in Great Ormond Street Hospital. We will reimburse any travelling expenses.

At the appointment we will ask you to do the following:

After the study has been explained to you (and we have answered all your questions) we would like you or your parent to sign a consent form. This will give us permission to include you in the study and to obtain information about genotype (your genetic code) and clinical details from the hospital notes.

We will measure your standing and sitting height and weight, waist, hip, head, arm and leg circumference and limb length, and skin-folds (by pressing the fat gently on the arm, shoulder and abdomen). You will not be asked to undress completely.

We will ask you to lie on a bed with a machine above (DXA scan) that measures bone using very low dose X-rays (much less than the daily background radiation we are all exposed to). This will also take a picture of your skeleton, which you can keep. The scans take a few minutes each, and are performed with you wearing light indoor clothing (such as shorts and a t-shirt) that do not contain metal. These scans give a measurement of the size and amount of mineral in the bones, as well as the proportions of fat and muscle. **If there is any possibility that the person being scanned is pregnant, the scan will not be performed.**

We will measure the strength of the bones in your wrist and lower leg using an ultrasound machine. The measurements take a few minutes, are completely painless and do not use X-rays.

We will measure the shape, size and bone mineral content of your tibia (lower leg bone) and radius (lower arm bone) using a pQCT machine. This uses a very low dose of X-rays (much less than the daily background radiation we are all exposed to).

We will measure the amount of water in your body. This measurement involves drinking some water containing heavy oxygen molecules (18-oxygen). These molecules are not radioactive, they simply weigh more than most oxygen molecules and they occur naturally in all of us. Before and 4 hours after the drink you will be asked to provide a saliva sample using an absorbent cotton wool swab.

We will measure the volume of your body. This measurement involves you sitting still inside a chamber (with a clear window) called a BodPod for about 2 minutes whilst room air is gently blown around you. This measurement will be repeated a second and possibly third time. The test is performed with you wearing a swimming costume and a polyester swimming hat (which we will provide).

We will perform a measurement of bioelectrical impedance using a low level of electrical current (that is undetectable), passed between electrodes in contact with your hands and feet. The test is harmless and painless and gives a measure of body water.

We will ask you some questions about general health, including any medicines being taken, and use a short questionnaire to measure calcium intake and activity level. To avoid undressing we will give you a leaflet to complete in private, that shows pictures of the different stages of pubertal development and ask you to indicate which is closest to you.

All the things we have asked you to do will take about 1 ½ hours. None of the tests will hurt.

j) To measure the amount of activity that you do, we will ask you to wear an activity monitor on a belt during the daytime for 7 days (5 week days and 2 week-end days). This machine can be posted back to us when the measurements are finished.

Are there any risks and discomforts?

All of the tests are painless and harmless. DXA and pQCT scans involve a tiny amount of radiation, which is less than half of a day's background radiation in the United Kingdom (to which we are all exposed), and less than one tenth of the radiation from a flight across the Atlantic.

What are the potential benefits?

The study could identify any abnormalities of bone density or in the proportions of fat and lean tissue. Where possible, these would be treated. Most people find the tests interesting and educational and everyone will be given a printout of his/her skeleton to take home.

Who will have access to the research records?

Only the researchers will have access to the data collected during this study.

Do I have to take part in this study?

No. If you decide, now or at a later stage, that you do not wish to take part in the study, that is entirely your right and it will not affect your clinical care.

What will happen to the results of the research study?

The results of this research will be published in a medical journal and presented at scientific meetings. The clinical team will receive the results of the bone density scan.

What if something goes wrong?

The research project has been approved by an Independent Research Ethics Committee which believes that it is of minimal risk to you. However, any research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this project.

This research is covered by a no-fault compensation scheme which may apply

in the event of any significant harm resulting from involvement in the project. Under this scheme it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or any manufacturer involved.

Who do I speak to if problems arise?

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact Mrs Jane Clist by post at the Research and Development Office, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, or, if urgent, by telephone on 020 7905 2201.


Details of how to contact the researchers:

Jane Williams
Research Nurse
Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2743
Email; jane.williams@ich.ucl.ac.uk

Dr Mary Fewtrell
Chief Investigator
Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2389
Email; m.fewtrell@ich.ucl.ac.uk

Thank-you for reading this information sheet!

Appendix 2.4. Control over 12y information sheet

Great Ormond Street 
Hospital for Children
NHS Trust



MRC Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel 020 7905 2389

Title of project

Body composition and bone mineralisation in children with cystic fibrosis compared to healthy children.

Participant Information Sheet (12 yrs and older).

You are being invited to take part in a research study. Before you decide whether to take part or not, it is important to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. You can find more general information about research on the Great Ormond Street Hospital website:

(http://www.gosh.nhs.uk/gosh_families/research)

What is the aim of the study?

The aim of this study is to measure the amount of fat, muscle and bone in children with cystic fibrosis (CF) and to see if these measurements relate to how well the children are. In order to do this we need to measure healthy children as a comparison.

Why is the study being done?

Some children with CF may develop problems with growth, due to problems with the absorption of food. This may lead to differences in the proportions of fat and muscle in the body, and also with the amount of mineral in their bones. Until recently, it has been difficult to accurately measure the amounts

of fat, muscle and bone in children, but we can now do this using some simple measurements that are available at GOSH.

How is the study to be done?

We are inviting healthy, full term children aged 8-19 years to participate. If you are willing to take part in the study, we will arrange an appointment at a time which is convenient for you. If you agree we will contact you again every 2 years until the age of 19 years to repeat the measurements. You can decide on each occasion whether to take part. The study will take about 1 ½ hours and will take place in Great Ormond Street Hospital. We will reimburse any travelling expenses.

At the appointment we will ask you to do the following:

After the study has been explained to you (and we have answered all your questions) we would like you or your parent to sign a consent form. This will give us permission to include you in the study.

We will measure your standing and sitting height and weight, waist, hip, head, arm and leg circumference and limb length, and skin-folds (by pressing the fat gently on the arm, shoulder and abdomen). You will not be asked to undress completely.

We will ask you to lie on a bed with a machine above (DXA scan) that measures bone using very low dose X-rays (much less than the daily background radiation we are all exposed to). This will also take a picture of your skeleton, which you can keep. The scans take a few minutes each, and are performed with you wearing light indoor clothing (such as shorts and a t-shirt) that does not contain metal. These scans give a measurement of the size and amount of mineral in the bones, as well as the proportions of fat and muscle. **If there is any possibility that the person being scanned is pregnant, the scan will not be performed.**

We will measure the strength of the bones in your wrist and lower leg using an ultrasound machine. The measurements take a few minutes, are completely painless and do not use X-rays.

We will measure the shape, size and bone mineral content of your tibia (lower leg bone) and radius (lower arm bone) using a pQCT machine. This uses a very low dose of X-rays (much less than the daily background radiation we are all exposed to).

We will measure the amount of water in your body. This measurement involves drinking some water containing heavy hydrogen molecules (deuterium). These molecules are not radioactive, they simply weigh more than most hydrogen molecules and they occur naturally in all of us. Before and 4 hours after the drink you will be asked to provide a saliva sample using an absorbent cotton wool swab.

We will measure the volume of your body. This measurement involves you sitting still inside a chamber (with a clear window) called a BodPod for about 2 minutes whilst room air is gently blown around you. This measurement will be repeated a second and possibly third time. The test is performed with you wearing a swimming costume and a polyester swimming hat (which we will provide).

We will perform a measurement of bioelectrical impedance using a low level of electrical current (that is undetectable), passed between electrodes in

contact with your hands and feet. The test is harmless and painless and gives a measure of body water.

We will ask you some questions about general health, including any medicines being taken, and use a short questionnaire to measure your calcium intake and activity level. To avoid undressing we will give you a leaflet to complete in private, that shows pictures of the different stages of pubertal development and ask you to indicate which is closest to you.

All the things we have asked you to do will take about 1 ½ hours. None of the tests will hurt you.

j) To measure the amount of activity that you do, we will ask you to wear an activity monitor on a belt during the daytime for 7 days (5 week days and 2 week-end days). This machine can be posted back to us when the measurements are finished.

Are there any risks and discomforts?

All of the tests are painless and will not harm you. DXA and pQCT scans involve a tiny amount of radiation, which is less than half of a day's background radiation in the United Kingdom (to which we are all exposed), and less than one tenth of the radiation from a flight across the Atlantic.

What are the potential benefits?

The tests that we will use are mainly a research tool. However, if we identify any problems, we will, with your permission, contact your GP so that further investigations or treatment can be arranged if appropriate.

Who will have access to the research records?

Only the researchers will have access to the data collected during this study.

Do I have to take part in this study?

No. If you decide, now or at a later stage, that you do not wish to take part in the study, that is entirely your right and it will not prejudice any present or future treatment.

What will happen to the results of the research study?

The results of this research will be published in a medical journal and presented at scientific meetings. The clinical team will receive the results of the bone density scan.

What if something goes wrong?

The research project has been approved by an Independent Research Ethics Committee which believes that it is of minimal risk to you. However, any research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this project.

This research is covered by a no-fault compensation scheme which may apply in the event of any significant harm resulting to you from involvement in the project. Under this scheme it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or any manufacturer involved.

Who do I speak to if problems arise?

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact Mrs Jane Clist by post at the Research and Development Office, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, or, if urgent, by telephone on 020 7905 2201.

Details of how to contact the researchers:

Jane Williams
Research Nurse
Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2743
Email; jane.williams@ich.ucl.ac.uk

Dr Mary Fewtrell
Chief Investigator
Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2389
Email; m.fewtrell@ich.ucl.ac.uk

Thank-you for reading this information sheet!

Appendix 2.5. Parent of child with CF information sheet

Great Ormond Street
Hospital for Children

NHS Trust



MRC Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel 020 7905 2389

Title of project

Body composition and bone mineralisation in children with Cystic Fibrosis compared to healthy children.

Parent Information Sheet

Your child is being invited to take part in a research study. Before you decide whether he/she should take part or not, it is important to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. You can find more general information about research on the Great Ormond Street Hospital website:

(http://www.gosh.nhs.uk/gosh_families/research)

What is the aim of the study?

The aim of this study is to measure the amount of fat, muscle and bone in children with cystic fibrosis (CF) and to see if these measurements relate to how well the children are.

Why is the study being done?

Some children with CF may develop problems with growth, due to problems with the absorption of food. This may lead to differences in the proportions of fat and muscle in the body, and also with the amount of mineral in their bones. Until recently, it has been difficult to accurately measure the amounts of fat, muscle and bone in children, but we can now do this using some simple measurements that are available at GOSH.

How is the study to be done?

We are inviting all children aged 6-17 years with CF who attend GOSH to participate. If you are willing to take part in the study, we will arrange an appointment at a time which is convenient for you (if possible, at the same time as your outpatient appointment). If you and your child agree we will contact you again, every 2 years to repeat the measurements. You can decide on each occasion whether to take part. The study will take about 1 ½ hours and will take place in Great Ormond Street Hospital. We will reimburse any travelling expenses.

At the appointment we will ask you to do the following:

After the study has been explained to you (and we have answered all your questions) we would like you to sign a consent form. This will give us permission to include your child in the study and to obtain information about genotype (the genetic code) and clinical details from the hospital notes. We will also ask for your permission to contact you again in two years time. We will measure your child's height, sitting height and weight, waist, hip, head, arm and leg circumference and limb length, and skin-folds (by pressing the fat gently on the arm, shoulder and abdomen). Children will not be asked to undress completely.

We will ask your child to lie on a bed with a machine above them (DXA scan) that measures bone using very low dose X-rays (much less than the daily background radiation we are all exposed to). This will also take a picture of your child's skeleton, which you can keep. The scans take a few minutes each, and are performed with your child wearing light indoor clothing (such as shorts and a t-shirt). These scans give a measurement of the size and amount of mineral in the bones, as well as the proportions of fat and muscle. **If there is any possibility that the person being scanned is pregnant, the scan will not be performed.**

We will measure the strength of the bones in your child's wrist and lower leg using an ultrasound machine. The measurements take a few minutes, are completely painless and do not use X-rays.

We will measure the shape, size and bone mineral content of the tibia (lower leg bone) and radius (lower arm bone) using a pQCT machine. This uses a very low dose of X-rays (much less than the daily background radiation we are all exposed to).

We will measure the amount of water in the body. This measurement involves drinking some water containing heavy oxygen molecules (18-oxygen). These molecules are not radioactive, they simply weigh more than most oxygen molecules and they occur naturally in all of us. Before and 4 hours after the drink your child will be asked to provide a saliva sample using an absorbent cotton wool swab.

We will measure the volume of the body. This measurement involves your child sitting still inside a chamber (with a clear window) called a BodPod for about 2 minutes whilst room air is gently blown around them. This measurement will be repeated a second and possibly a third time. The test is performed with the child wearing a swimming costume and a polyester swimming hat (which we will provide).

We will perform a measurement of bioelectrical impedance using a low level of electrical current (that is undetectable), passed between electrodes in

contact with the hands and feet. The test is harmless and painless and gives a measure of body water.

We will ask you and your child some questions about general health, including any medicines being taken, and use a short questionnaire to measure his or her calcium intake and activity level. For older children (above about 9 years of age) we also need to know if any pubertal development has taken place as this affects the growth and mineral content of bones. To avoid any undressing, we will show your child some pictures of the different stages of pubertal development (in private) and ask him or her to pick the one which is closest to them.

All the things we have asked your child to do will take about 1 ½ hours. None of the tests will hurt your child.

j) To measure the amount of activity that your child does, we will ask him or her to wear an activity monitor on a belt during the daytime for 7 days (5 week days and 2 week-end days). This machine can be posted back to us when the measurements are finished.

Are there any risks and discomforts?

All of the tests are painless and will not harm your child. DXA and pQCT scans involve a tiny amount of radiation, which is less than half of a day's background radiation in the United Kingdom (to which we are all exposed), and less than one tenth of the radiation from a flight across the Atlantic.

What are the potential benefits?

The study could identify any abnormalities of bone density or in the proportions of fat and lean tissue. Where possible, these would be treated. Most children find the tests interesting and educational and each child will be given a printout of his/her skeleton to take home.

Who will have access to the research records?

Only the researchers will have access to the data collected during this study.

Does my child have to take part in this study?

No. If you decide, now or at a later stage, that you do not wish to take part in the study, that is entirely your right and it will not affect your child's clinical care.

What will happen to the results of the research study?

The results of this research will be published in a medical journal and presented at scientific meetings. The clinical team will receive the results of the bone density scan.

What if something goes wrong?

The research project has been approved by an Independent Research Ethics Committee which believes that it is of minimal risk to your child. However, any research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this project.

This research is covered by a no-fault compensation scheme which may apply in the event of any significant harm resulting to your child from involvement in the project. Under this scheme it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or any manufacturer involved.

Who do I speak to if problems arise?

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact Mrs Jane Clist at the Research and Development Office, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, or, if urgent, by telephone on 020 7905 2201.

Details of how to contact the researchers:

Jane Williams
Research Nurse
Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
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Tel: 0207 905 2743
Email; jane.williams@ich.ucl.ac.uk

Dr Mary Fewtrell
Chief Investigator
Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2389
Email; m.fewtrell@ich.ucl.ac.uk

Thank-you for reading this information sheet!

Appendix 2.6. Parent of control child information sheet

Great Ormond Street 
Hospital for Children
NHS Trust



MRC Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel 020 7905 2389

Title of project

Body composition and bone mineralisation in children with Cystic Fibrosis compared to healthy children.

Parent Information Sheet

Your child is being invited to take part in a research study. Before you decide whether he/she should take part or not, it is important to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. You can find more general information about research on the Great Ormond Street Hospital website:

(http://www.gosh.nhs.uk/gosh_families/research)

What is the aim of the study?

The aim of this study is to measure the amount of fat, muscle and bone in children with cystic fibrosis (CF) and to see if these measurements relate to how well the children are. In order to do this we need to measure healthy children as a comparison.

Why is the study being done?

Some children with CF may develop problems with growth, due to problems with the absorption of food. This may lead to differences in the proportions of fat and muscle in the body, and also with the amount of mineral in their bones. Until recently, it has been difficult to accurately measure the amounts of fat, muscle and bone in children, but we can now do this using some simple measurements that are available at GOSH.

How is the study to be done?

We are inviting healthy, full term children aged 8-19 years to participate. If you are willing to take part in the study, we will arrange an appointment at a time which is convenient for you. If you and your child agree we will contact you again, every 2 years until your child is 19 years old to repeat the measurements. You can decide on each occasion whether to take part. The study will take about 1 ½ hours and will take place in Great Ormond Street Hospital. We will reimburse any travelling expenses.

At the appointment we will ask you to do the following:

After the study has been explained to you (and we have answered all your questions) we would like you to sign a consent form. This will give us permission to include your child in the study and to contact you again in two years time.

We will measure your child's height, sitting height and weight, waist, hip, head, arm and leg circumference and limb length, and skin-folds (by pressing the fat gently on the arm, shoulder and abdomen). Children will not be asked to undress completely.

We will ask your child to lie on a bed with a machine above them (DXA scan) that measures bone using very low dose X-rays (much less than the daily background radiation we are all exposed to). This will also take a picture of your child's skeleton, which you can keep. The scans take a few minutes each, and are performed with your child wearing light indoor clothing (such as shorts and a t-shirt). These scans give a measurement of the size and amount of mineral in the bones, as well as the proportions of fat and muscle. **If there is any possibility that the person being scanned is pregnant, the scan will not be performed.**

We will measure the strength of the bones in your child's wrist and lower leg using an ultrasound machine. The measurements take a few minutes, are completely painless and do not use X-rays.

We will measure the shape, size and bone mineral content of the tibia (lower leg bone) and radius (lower arm bone) using a pQCT machine. This uses a very low dose of X-rays (much less than the daily background radiation we are all exposed to).

We will measure the amount of water in the body. This measurement involves drinking some water containing heavy hydrogen molecules (deuterium). These molecules are not radioactive, they simply weigh more than most hydrogen molecules and they occur naturally in all of us. Before and 4 hours after the drink your child will be asked to provide a saliva sample using an absorbent cotton wool swab.

We will measure the volume of the body. This measurement involves your child sitting still inside a chamber (with a clear window) called a BodPod for about 2 minutes whilst room air is gently blown around them. This measurement will be repeated a second and possibly a third time. The test is performed with the child wearing a swimming costume and a polyester swimming hat (which we will provide).

We will perform a measurement of bioelectrical impedance using a low level of electrical current (that is undetectable), passed between electrodes in contact with the hands and feet. The test is harmless and painless and gives a measure of body water.

We will ask you and your child some questions about general health, including any medicines being taken, and use a short questionnaire to measure his or her calcium intake and activity level. For older children (above about 9 years of age) we also need to know if any pubertal development has taken place as this affects the growth and mineral content of bones. To avoid any undressing, we will show your child (in private) some pictures of the different stages of pubertal development and ask him or her to pick the one which is closest to them.

All the things we have asked your child to do will take about 1 ½ hours. None of the tests will hurt your child.

j) To measure the amount of activity that your child does, we will ask him or her to wear an activity monitor on a belt during the daytime for 7 days (5 week days and 2 week-end days). This machine can be posted back to us when the measurements are finished.

Are there any risks and discomforts?

All of the tests are painless and will not harm your child. DXA and pQCT scans involve a tiny amount of radiation, which is less than half of a day's background radiation in the United Kingdom (to which we are all exposed), and less than one tenth of the radiation from a flight across the Atlantic.

What are the potential benefits?

The tests that we will use are mainly a research tool. However, if we identify any problems, we will, with your permission, contact your child's GP so that further investigations or treatment can be arranged if appropriate.

Who will have access to the research records?

Only the researchers will have access to the data collected during this study.

Does my child have to take part in this study?

No. If you decide, now or at a later stage, that you do not wish to take part in the study, that is entirely your right and it will not prejudice any present or future treatment.

What will happen to the results of the research study?

The results of this research will be published in a medical journal and presented at scientific meetings. The clinical team will receive the results of the bone density scan.

What if something goes wrong?

The research project has been approved by an Independent Research Ethics Committee which believes that it is of minimal risk to your child. However, any research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this project.

This research is covered by a no-fault compensation scheme which may apply in the event of any significant harm resulting to your child from involvement in the project. Under this scheme it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or any manufacturer involved.

Who do I speak to if problems arise?

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact Mrs Jane Clist at the Research and Development Office, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, or, if urgent, by telephone on 020 7905 2201.

Details of how to contact the researchers:

Jane Williams
Research Nurse
Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2743
Email; jane.williams@ich.ucl.ac.uk

Dr Mary Fewtrell
Chief Investigator
Childhood Nutrition Research Centre
Institute of Child Health
30 Guilford Street
London WC1N 1EH
Tel: 0207 905 2389
Email; m.fewtrell@ich.ucl.ac.uk

Thank-you for reading this information sheet!

Appendix 3. Appointment letter

UCL INSTITUTE OF CHILD HEALTH

Name and address
27 August 2013

Dear,

Appointment for body composition study on Tuesday 14th April at 11.30am.

Thank you for volunteering to take part in the study of body composition at Great Ormond Street Hospital. If you enter the main entrance of the hospital in Gt Ormond Street I will meet you at the X ray reception which is situated on the same level and in the Variety Club Building. Tell X ray reception that you are booked in for a DEXA scan.

If you have any problems attending this appointment please call and leave me a message, tel: 020 7905 2743 and I will organise another date. If you need to contact me on the day, then ring the hospital switchboard, tel: 020 7405 9200 and ask them for extension 2309 or 0559.

The bone density scan can be performed whilst wearing light clothing providing there are **no metal zips, buttons, bra hooks etc.** In order to do an accurate measurement of body volume in the Bodpod, it is necessary to wear a **close fitting swimming costume**. Loose fitting shorts will affect the measurement and are not acceptable but tight fitting shorts are acceptable. If you think you may have a problem let me know before the appointment.

Please keep your travel tickets for re-imbursement on the day. The nearest underground stations are Russell Sq and Holborn.

Please call me if you have any questions at all.

Yours sincerely,

Jane Williams
Research Nurse
Tel: 020 7905 2743
Email: jane.williams@ich.ucl.ac.uk

Enc; Map and Information sheet

Appendix 4.1. Child assent form



ASSENT FORM

Title of the Research project:

Body composition and bone health in children with cystic fibrosis

Sponsor Protocol No: 02NT01

Investigator: Dr Mary Fewtrell

Contact details:  : 0207 905 2389/2251  : m.fewtrell@ich.ucl.ac.uk

Subject Identification No for this trial: _____

Please initial box
to indicate agreement:

1	I have read and understand the information sheet dated 02/05/08 (version 1) for the above study and have had the chance to ask questions.	
2	I understand that taking part is voluntary and that I can decide not to take part at any time.	
3	I agree to take part in the above study.	

Name of young person Date Signature _____

Name of Person taking consent Date Signature _____
(if different from Investigator)

Investigator Date Signature _____

Appendix 4.2. Participant consent form



PARTICIPANT CONSENT FORM

Title of the Research project:

Body composition and bone health in children with cystic fibrosis

Sponsor Protocol No: 02NT01

Investigator: Dr Mary Fewtrell

Contact details:  :0207 905 2389/2251  : m.fewtrell@ich.ucl.ac.uk

Subject Identification No for this trial: _____

Please initial box
to indicate agreement:

1	I confirm that I have read and understand the information sheet dated 02/05/08 (version 1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3	I understand that relevant sections of any of my Medical Notes and data collected during the study, may be looked at, by employees from Regulatory Authorities or from Great Ormond Street Hospital/ Institute of Child Health, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
4	I agree to my GP being informed of any sub-optimal results noted as a result of participating in this study.	
5	I agree to take part in the above study and I understand that I may be contacted in the future regarding re-measurement. I will be able to decide at that time whether to take part and give consent.	

Name of Participant

Date

Signature

Name of Person taking consent
(if different from Investigator)

Date

Signature

Investigator

Date

Signature



Appendix 4.3. Parent consent form

PARENT/GUARDIAN CONSENT FORM

Title of the Research project:

Body composition and bone health in children with cystic fibrosis

Sponsor Protocol No: 02NT01 Investigator: Dr Mary Fewtrell

Contact details:  :0207 905 2389/2251  : m.fewtrell@ich.ucl.ac.uk

Subject Identification No for this trial: _____

Please initial box
to indicate agreement:

1	I confirm that I have read and understand the information sheet dated 02/05/08 (version 1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2	I understand that my child's participation is voluntary and that he/she is free to withdraw at any time, without giving any reason, without his/her medical care or legal rights being affected.	
3	I understand that relevant sections of any of my child's Medical Notes and data collected during the study, may be looked at, by employees from Regulatory Authorities or from Great Ormond Street Hospital/ Institute of Child Health, where it is relevant to my child's taking part in this research. I give permission for these individuals to have access to my child's records.	
4	I agree to my child's GP being informed of any sub-optimal results noted as a result of participating in this study.	
5	I agree to my child taking part in the above study and I understand that I may be contacted in the future regarding re-measurement. I will be able to decide at that time whether to take part and give consent.	

Name of Child

Name of Parent/Guardian

Date

Signature

Name of Person taking consent
(if different from Investigator)

Date

Signature

Investigator

Date

Signature

[illegible]258



2006

BODY COMPOSITION: PATIENT GROUP (CF) Id C F /

Social Data cont'd

Father's date of birth / / 1 9

1. No. of quals	2. <3 OSEs or GCSE's below G grade
3. >3 OSEs or any O levels or GCSE grade A-C	
4. A levels, ONC/OND/BE/TEC, SCE Higher, NVA level 3	
5. Degree/HND/HNC, professional training (incl SHINCON/VA/VAH), NVQ levels	
6. BEC/TEC Higher	

Father's educational attainments (highest completed) Father's highest qualification
 Father's occupation
 How many months employed in the last year? Who is the primary earner for the family? ☐ Father☐ Mother☐ Grandparent☐ Both parents☐ MissingSocial class (use primary earner's occupation) Social code as below

Code as: 1 = 1, 2 = 2, 3N = 3, 3M = 4, 4 = 5, 5 = 6 on primary earner's occupation, Single parent mother unsupported and not working = 7 Mother supported but partner and self never employed = 8 Adopted / fostered child = 9

Page 1.2



2006

BODY COMPOSITION: PATIENT GROUP (CF) Id C F /

Social Data cont'd

How many children live in the family home? How many are mother's own children?

Ethnic origin

Please tick the box that you think best describes your ethnic origin

- White ☐
- Black-Caribbean ☐
- Black-African ☐
- Black-other ☐
- Indian ☐
- Pakistani ☐
- Bangladeshi ☐
- Chinese ☐
- Asian-other ☐
- Other ☐
- Refused ☐

Page 1.3

Id	C	F			
----	---	---	--	--	--

Hospital ID							
-------------	--	--	--	--	--	--	--

GENOTYPE

FEV ₁	% predicted
1	

☐ YES=1, NO=2

OR OTHER? ☐ YES=1 NO=2

DESCRIBE

[illegible]

Dietary Therapy

Overnight Feed? ☐ YES=1, NO=2

PANCREATIC ENZYME	CAPSULES PER DAY

Id	C	F			
----	---	---	--	--	--

Fractures

Ever had a fracture? ☐ yes=1, no=2 if no, turn to next section

First accident, bones fractured

01=numerus 05=iltria 09=skull 13=pelvis 17=other
02=radius 06=fibula 10=vertebrae 14=scapula
03=ulna 07=hand 11=ribs 15=pelvis
04=femur 08=foot 12=clavicle 16=sternum

[illegible]

Cause of first accident

01=fall playground apparatus 04=RTA in car 07=injury at home
02=fall from bike 05=RTA pedestrian 08=other
03=RTA bike 06=sports injury

[illegible]

Place where first accident happened

01=home	03=street
02=school	04=other

[illegible]



10	C	E			
----	---	---	--	--	--

Second accident, bones fractured

01=numberus	05=alba	09=skull	13=palvis	99=other
02=radius	06=ibula	10=vertebrae	14=scapula	
03=ulna	07=hand	11=ribs	15=pallia	

fracture 1		which side		other	
fracture 2		which side		other	
fracture 3		which side		other	

$$-2\alpha\beta^2 - \beta^2 = -2\alpha\beta^2 - \beta^2$$

01=fall playground apparatus 04=RTA in car 07=injury at home
02=fall from bike 05=RTA pedestrian 08=other
03=RTA bike 06=sports injury

[illegible]

10

01=home 03=street/road
02=school 04=other

[illegible]

id	0	1	2
----	---	---	---

Third accident, bones fractured

01=humeros
02=radius
03=ulna
04=femur
05=iliza
06=huila
07=hend
08=foot
09=skull
10=vertebrae
11=ribs
12=clavicle
13=ovis
14=scapula
15=pelvis
16=sternum
17=otus

[illegible]

— 210 —

01=fall playground apparatus	04=RTA in car	07=injury at home
02=fall from bike	05=RTA pedestrian	08=other
03= RTA bike	06=sports injury	

[illegible]

1

01=home 03=street/road
02=school 04=other

[illegible]



BODY COMPOSITION: PATIENT GROUP (CF)

Id ☐ C ☐ F ☐ ☐

Vitamin and mineral supplements

Taken calcium supplements? ☐ yes
☐ no

name of calcium supplement

date started / / date stopped / /

no capsules/day still taking calcium supplements
☐ yes
☐ no

Calcium intake (mg)/day derived from Caquest analysis

Multivitamins ☐ yes
☐ no

name of multivitamin

date started / / date stopped / /

no capsules/day still taking multivitamins
☐ yes
☐ no



BODY COMPOSITION: PATIENT GROUP (CF)

Id ☐ C ☐ F ☐ ☐

Family history of other disease:

With respect to subject (not parent), code 0 for no disease:

1 - mother 2 - father
3 - brother 4 - sister
5 - maternal grandmother 6 - maternal grandfather
7 - paternal grandmother 8 - paternal grandfather

High Cholesterol

Heart disease

Osteoporosis

Hypertension

Diabetes - non-insulin dependant

Diabetes - insulin dependant

CF

Ever required surgery for any of the following:
(1 = yes, 2 = no)

Lung heart GI tract liver kidney

Please give details of any surgery

Ever required medical treatment for any of the following:
(1 = yes, 2 = no)

Lung heart GI tract liver kidney

Please give details of any medical treatment

ROUP (CF)

Id	C	F	.			
----	---	---	---	--	--	--

Hospital ID

--	--	--	--	--	--	--

Id	C	F	.		
----	---	---	---	--	--

How long is each PE lesson, in minutes

--	--	--

How many hours per week do you spend watching TV and videos, plus playing on the computer and video games (weekend)

--	--	--	--

Riding bike

Tennis

Running	Hockey
<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>

[illegible]

Activity	1	2	3	4	5
Gymnastics					
Skating					

er, please specify

CF Study: Exercise/puberty (1 of 2)

Id	C	F	.			
----	---	---	---	--	--	--

Total hours child spends in vigorous activity per week

1 = much less than peers
2 = less
3 = same
4 = more
5 = much more

data to transfer from confidential data sheet

Public hair development (1-5)			
-------------------------------	--	--	--

Have periods started ☐ yes ☐ no ☐ not applicable

Oral contraceptive or implant? ☐ ☐ ☐

Number of days in whole menstrual cycle

CF Study: Exercise/puberty (2 of 2)



3/8/16

BODY COMPOSITION STUDY: PATIENT GROUP (CF)Id ☐ C ☐ F ☐ ☐Hospital ID ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐Gender ☐ 1=male
2=femaleGestation ☐ ☐ ☐Birth Weight (g) ☐ ☐ ☐ ☐ ☐ ☐Mother's reported weight ☐ ☐ ☐ ☐ ☐ ☐Mother's reported height ☐ ☐ ☐ ☐ ☐ ☐Father's reported weight ☐ ☐ ☐ ☐ ☐ ☐Father's reported height ☐ ☐ ☐ ☐ ☐ ☐**Anthropometry**MUAC (cm) ☐ ☐ ☐ ☐ ☐ ☐Head circum (cm) ☐ ☐ ☐ ☐ ☐ ☐Waist circum (cm) ☐ ☐ ☐ ☐ ☐ ☐Hips (cm) ☐ ☐ ☐ ☐ ☐ ☐Thigh circum (cm) ☐ ☐ ☐ ☐ ☐ ☐Calf circum (cm) ☐ ☐ ☐ ☐ ☐ ☐**Skinfold Thickness Measurements**Biceps (mm) ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐Triceps skin-fold (mm) ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐Subscap (mm) ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐Suprailiac (mm) ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐

Page 4.1



3/8/16

BODY COMPOSITION STUDY: PATIENT GROUP (CF)Id ☐ C ☐ F ☐ ☐**BODPOD**Date ☐ ☐ / ☐ ☐ / ☐ ☐Time ☐ ☐ ☐ ☐ Temp ☐ ☐ ☐ ☐Pressure ☐ ☐ ☐ ☐**Final Test System**SD ☐ ☐ Mean Volume ☐ ☐ ☐ ☐**Test Data**

Raw Volume Test One Test Two Test Three Test Four

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Page 4.2



BODY COMPOSITION STUDY: PATIENT GROUP Id C F
(CF)

DEUTERIUM DILUTION

Full bottle of diluted deuterium (g)
Post dosing bottle weight (g)

Post dose fluid intake (mls)
Time post dose saliva sample taken

BIOELECTRICAL IMPEDANCE

	Log no.	distance (cm)
Whole arm	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Lower arm	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Upper arm	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Trunk	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Upper leg	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Whole leg	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Lower leg	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Whole body	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Whole body	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>

[illegible]

12895

BODY COMPOSITION: REFERENCE DATA

Id /

Social Data

Today's date

/ /

Unique file identifier

Uses own pencil, number of lines and final 2 letters + 3 digit study number e.g. 111110X225

Number of people in home (incl subject)

Number of rooms in home (incl dining, living + bedrooms, excl kitchen + bathroom)

Is home own or rented? ☐ Owned ☐ Rented

If home is rented, is it from: ☐ Private landlord ☐ Council ☐ Housing association

Is mother married?

☐ Yes ☐ No ☐ Irrelevant

Living with partner

Mother's date of birth / /

1. Name (surname, first names, initials) of mother, as recorded on birth certificate.
 2. Date of birth of mother, as recorded on birth certificate.
 3. Sex of mother, as recorded on birth certificate.
 4. Is mother married?
 5. Is mother living with partner?
 6. Number of people in home (including subject).
 7. Number of rooms in home (including dining, living + bedrooms, excluding kitchen + bathroom).
 8. Is home owned or rented?
 9. If home is rented, is it from:
 10. Unique identifier (uses own pencil, number of lines and final 2 letters + 3 digit study number e.g. 111110X225).

Mother's educational attainments (highest completed)

Mother's highest qualification

Mother's occupation

How many months employed in the last year?

Page 1, 1



52702

BODY COMPOSITION STUDY: REFERENCE DATA

☐ ☒ ☐ ☐ ☐

Fractures continued

Third accident, bones fractured

01=hip 03=thigh 09=skull 13=pelvis 17=other
02=femur 06=forearm 10=vertebrae 14=scapula
05=ulna 07=hand 11=rib 15=prolifer
04=thumb 08=foot 12=clavicle 16=sternum

fracture 1				which side		other														
fracture 2				which side		other														
fracture 3				which side		other														

Cause of third accident

01=fall playground apparatus 06=fall from car 07=falling at home
02=fall from bike 08=fall from stairs 09=fall from
03=fall from bike 04=fall from stairs 05=fall from stairs

cause fracture 1				other																
cause fracture 2				other																
cause fracture 3				other																

Place where third accident happened

01=home 03=street/road
02=playground 04=other

fracture 1				other																
fracture 2				other																
fracture 3				other																



52702

BODY COMPOSITION STUDY: REFERENCE DATA

☐ ☒ ☐ ☐ ☐

Medical Details

Has child had a significant illness during childhood? ☐ yes=1, no=2
If no, continue to next section

Type of illness

01=asthma 04=genital disease
02=diabetes 03=other
05=epilepsy

illness 1 type				duration (yrs)																
illness 2 type				duration (yrs)																
illness 3 type				duration (yrs)																

if other illness please state

illness 1																				
illness 2																				
illness 3																				

Has child been on long term medication/currently taking medication? ☐
(yes=1, no=2 if no, turn to next section)

Type of medication

01=antibiotic 06=cardiacs 11=epilepsy
02=antidepressant 07=endocrine 12=sedative
03=antipsychotic 08=eye medicine 13=analgesic
04=antacid 09=hypertension 14=anticoagulant
05=exocrine pancreas/enzyme 10=immunization 15=other

drug 1																				
name																				
type																				
doseage																				
date started																				
date stopped																				
time on medication																				
frequency																				
still taking medication																				
yes																				
no																				



D	X			
---	---	--	--	--

Vitamin and mineral supplements

Calcium supplements ☐ yes ☐ no if no, continue to next section

date started / / date stopped / /

no capsules/day / /

Calcium intake (mg)/day	derived from Caiaquest analysis

name of multivitamin							
date started				date stopped			
[]	[]	/	[]	[]	[]	/	[]
no capsules/day [] []							
still taking multivitamins							
o yes							
o no							

D	X			
---	---	--	--	--

With respect to subject (not parent), code 0 for no disease:

With respect to subject (not parent), code 0 for no disease:

- | | |
|--------------------------|--------------------------|
| 1 - mother | 2 - father |
| 3 - brother | 4 - sister |
| 5 - maternal grandmother | 6 - maternal grandfather |
| 7 - paternal grandmother | 8 - paternal grandfather |

High Cholesterol

Heart disease

Osteoporosis

Hypertension

Diabetes - non-insulin dependant

Diabetes - insulin dependant

Has your child ever required surgery for any of the following:

(1 = yes, 2 = no)

lung

heart

GI tract

liver

kidney

Please give details of any surgery in childhood

[illegible][illegible]

(1 = yes, 2 = no)

lung

heart

GI tract

liver

kidney

Please give details of any medical treatment in childhood

[illegible][illegible]

Page 2.7

ID	D	X	.			
----	---	---	---	--	--	--

Children's Exercise Questionnaire

How many PE lessons do you have per week

How long is each PE lesson, in minutes

How many hours per week do you spend watching TV and videos, plus playing on the computer and video games (school nights only)

•

How many hours per week do you spend watching TV and videos, plus playing on the computer and video games (weekend)

--	--	--	--

How many hours per week do you spend on each of these activities outside PE classes:

Riding bike ☐ ☐ ☐ ☐ ☐

Swimming	Netball
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

Running

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Hockey

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Football

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Basketball

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	Aerobics/dancing	Rugby
1	<input type="checkbox"/>	<input type="checkbox"/>
2	<input type="checkbox"/>	<input type="checkbox"/>
3	<input type="checkbox"/>	<input type="checkbox"/>
4	<input type="checkbox"/>	<input type="checkbox"/>
5	<input type="checkbox"/>	<input type="checkbox"/>
6	<input type="checkbox"/>	<input type="checkbox"/>
7	<input type="checkbox"/>	<input type="checkbox"/>
8	<input type="checkbox"/>	<input type="checkbox"/>
9	<input type="checkbox"/>	<input type="checkbox"/>
10	<input type="checkbox"/>	<input type="checkbox"/>
11	<input type="checkbox"/>	<input type="checkbox"/>
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14	<input type="checkbox"/>	<input type="checkbox"/>
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46	<input type="checkbox"/>	<input type="checkbox"/>
47	<input type="checkbox"/>	<input type="checkbox"/>
48	<input type="checkbox"/>	<input type="checkbox"/>
49	<input type="checkbox"/>	<input type="checkbox"/>
50	<input type="checkbox"/>	<input type="checkbox"/>

Gymnastics

Walking .

Other, please specify



5452

BODY COMPOSITION STUDY: REFERENCE DATA

ID ☐ ☒ ☐ ☐ ☐

Parent's opinion of the child's physical activity levels

Total hours child spends in vigorous activity per week

Level of child's activity compared to peers

1 = Much less than peers
2 = Less than peers
3 = About same
4 = More than peers
5 = Much more

Pubertal Development (if 9 or above)

data to transfer from confidential data sheet

Breast/genital development stage (1-5)

Pubic hair development (1-5)

Age periods commenced (months)

Have periods started yes ☐

no ☐

not applicable ☐

Oral contraceptive or implant? Yes = 1
No = 2

Date of first day of last menstrual period / /

Number of days in whole menstrual cycle

For example: 5 days menstruation then 25 days to next menstruation = 30 day cycle.

Reference Study: Eorchiwubady (2 of 2)



988

BODY COMPOSITION STUDY: REFERENCE DATA

Id ☐ ☒ ☐ ☐ ☐
Unique ID ☐ ☐ ☒ ☐ ☐

Gender ☐ 1=male
2=female

Gestation

Birth Weight (g)

Mother's reported weight Mother's reported height

Father's reported weight Father's reported height

Anthropometry

MLUAC (cm)

Head circum (cm)

Waist circum (cm)

Hips (cm)

Thigh circum (cm)

Calf circum (cm)

Skinfold Thickness Measurements

Biceps (mm)

Triceps skin-fold (mm)

Subscap (mm)

Suprailiac (mm)



BODY COMPOSITION STUDY: REFERENCE DATA

Id

BODPOD

Date / /

Time : :

Temp

Pressure

Final Test System

SD

Mean Volume

Test Data

Raw Volume

Test One

Test Two

Test Three

Base Mean

Weight 1

Weight 2

Weight 3

% Fat 1

% Fat 2

% Fat 3

Raw Density (weight/volume)

Height

Lung Volume

Comments



BODY COMPOSITION STUDY: REFERENCE DATA

Id

DEUTERIUM DILUTION

Full bottle of diluted deuterium (g)

Post dosing bottle weight (g)

Post dose fluid intake (mls)

Time post dose saliva sample taken

BIOELECTRICAL IMPEDANCE

Whole arm

Log no.

distance (cm)

Lower arm

Upper arm

Trunk

Upper leg

Whole leg

Lower leg

Whole body

Whole body

Appendix 6. Saliva collection information

INSTRUCTIONS FOR TAKING SALIVA SAMPLE

Study No.

Date

We need you to take a sample of saliva after you get home and post it back to us in the envelope provided.

..... hours after your special drink, atpm we would like you to do another saliva sample in the same way you did the first. Do not eat, drink or clean your teeth for 30 minutes before taking the sample. Move the swab around your mouth until it is very wet without chewing on it.

It is very important that you take the sample at this time but if it is not possible it is better that you tell us the time it was taken.

Record time sample taken here.....

We also need to know how much you drank in the period between the special drink and the saliva sample you have done at home.

Record the amount of drink you have had:

.....cups

.....mugs

.....small glasses

.....large glasses

.....cans (330ml)

.....bottles (500ml)

Place the cotton swab in the tube and replace the lid firmly. Put the tube in the plastic bag and seal carefully.

Please return this sheet and the sample in the envelope provided as soon as possible.
THANK YOU FOR TAKING PART IN THIS STUDY

Jane Williams
Research Nurse
MRC Childhood Nutrition Research Centre
Institute of Child Health
30, Guilford Street
London WC1N 1EH
020 7905 2743
jane.williams@ich.ucl.ac.uk

Appendix 7. Certificate

CERTIFICATE OF APPRECIATION

Awarded to

John Smith

in recognition of valuable contributions to
research into body composition of children
and young people.



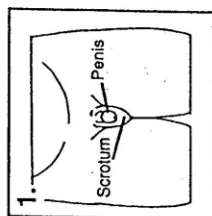
Childhood Nutrition Research Centre, Institute of Child Health
and Great Ormond Street Hospital for Children NHS Trust.

Signature.....Date.....

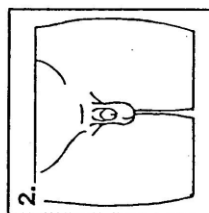
Appendix 8.1. Boys puberty questionnaire

Study Subject No:

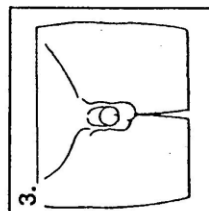
- Please look at the **Penis and Scrotum** only in these pictures.
- Please put a tick in the box that looks most like you now.



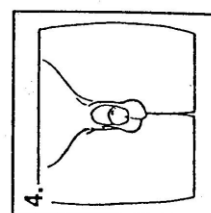
Scrotum and Penis same size as when you were younger.



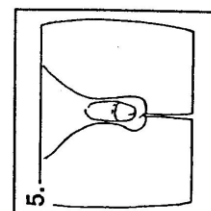
The Scrotum has lowered a bit and the Penis is a little larger.



The Penis is longer the Scrotum is larger.

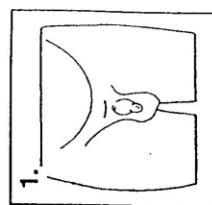


The Penis is longer and wider the Scrotum is darker and bigger than before

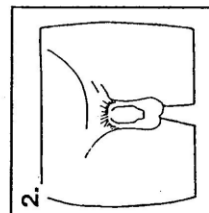


The Penis and Scrotum are the size and shape of an adult.

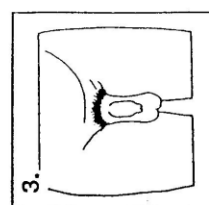
- Please look at the **Pubic Hair** only in these pictures.
- Please put a tick in the box that looks most like you now.



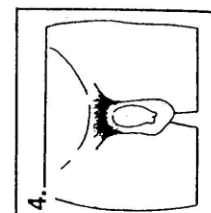
No hairs



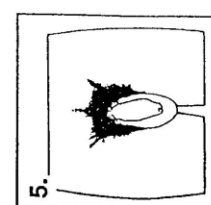
Very little hair



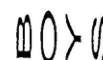
Quite a lot of hair



The hair has not spread over the thighs



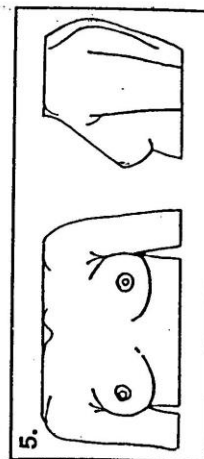
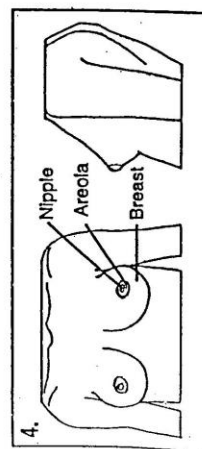
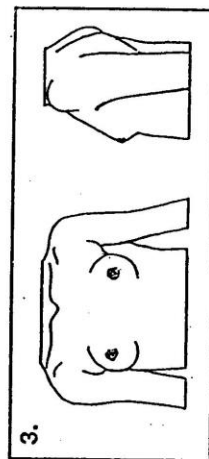
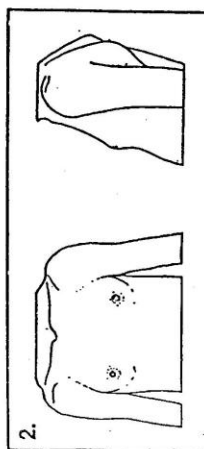
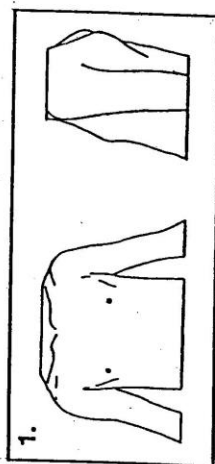
The hair has spread over the thighs



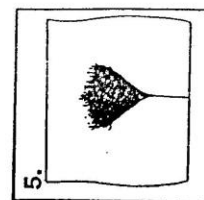
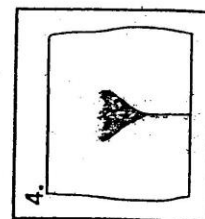
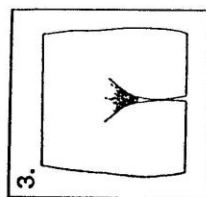
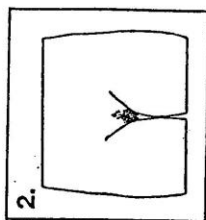
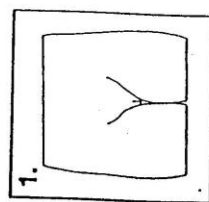
Appendix 8.2. Girls puberty questionnaire

Study Subject No:

- Please put a tick in the box that looks most like you now....



- Please put a tick in the box that looks most like you now....



Self Assessment

Self Assessment

Appendix 9. Comparison between children with CF that have dropped-out and those remaining in study after 2 years

	Drop-outs						Remaining in study			
	Boys			Girls			Boys		Girls	
	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>	Mean	SD	Mean	SD
Age	8.85	1.76	NS	9.99	2.15	NS	8.99	1.32	9.36	1.50
Height SDS	-0.07	0.80	NS	-0.78	1.45	NS	-0.55	1.02	-0.62	1.09
Weight SDS	0.62	1.30	NS	-0.87	0.97	NS	-0.21	1.05	-0.58	1.06
BMI SDS	0.83	1.68	NS	-0.57	0.46	NS	0.18	0.94	-0.35	1.09
FEV ₁ SDS	-0.73	2.06	NS	-2.36	1.29	NS	-0.21	1.46	-1.64	1.49

Appendix 10. Regression of factors impacting on waist circumference.

Regression analysis of effect of body composition, age, sex and height on waist circumference with sex as male = 0, female =1.

Children with CF;

Waist circumference = 67.518 + 1.224 (FM) + 0.838 (FFM) + 0.625 (age) – 2.303 (female) – 28.408 (height)

Adjusted $R^2 = 0.858$

Controls;

Waist circumference = 61.405 + 1.260(FM) + 0.690(FFM) – 0.254 (age) – 1.249(female) – 18.029(height)

Adjusted $R^2 = 0.913$

Partial correlations of the relationship between waist circumference and fat mass or fat-free mass or age or sex or height each adjusted for the others.

Partial correlation (adjusted for all other factors)	CF		Controls	
	R	P	R	P
Waist circumference and fat mass	0.817	<0.001	0.901	<0.001
Waist circumference and fat-free mass	0.533	<0.001	0.604	<0.001
Waist circumference and age	0.254	0.022	-0.096	0.392
Waist circumference and sex	-0.415	<0.001	-0.262	0.018
Waist circumference and height	-0.414	<0.001	-0.301	0.006

Appendix 11. Regression analyses for the relationship between baseline FEV₁ and change in body composition and baseline body composition and change in FEV₁.

		Boys			Girls		
IV	DV	B	SEM	P	B	SEM	P
Baseline FMI SDS	Change FEV ₁ SDS	-0.28	0.17	0.120	0.01	0.19	0.950
Baseline FFMI SDS	Change FEV ₁ SDS	0.17	0.16	0.191	0.24	0.17	0.181
Baseline FEV ₁	Change FMI SDS	-0.12	0.08	0.141	-0.05	0.08	0.589
Baseline FEV ₁	Change FFMI SDS	0.18	0.07	0.023	-0.01	0.06	0.851
Baseline FMI SDS	Conditional change FEV ₁ SDS	-0.25	0.17	0.149	0.08	0.18	0.662
Baseline FFMI SDS	Conditional change FEV ₁ SDS	0.18	0.16	0.258	0.22	0.16	0.170
Baseline FEV ₁ SDS	Conditional change FMI SDS	-0.11	0.07	0.146	-0.02	0.09	0.827
Baseline FEV ₁ SDS	Conditional change FFMI SDS	0.18	0.07	0.020	-0.01	0.06	0.557

Appendix 12. Bland-Altman analyses for simpler body composition techniques compared to the 4-component model.

Figure 1 (a-c) shows the results of the Bland-Altman analysis for DXA (whole body and regional), SFTs and BMI for predicting 4C FM SDS, and for DXA (whole body and regional) FMI for predicting 4C FMI SDS. Using the same analyses, Figure 2 (a-b) shows the assessment for DXA, TBW and BIA to predict 4C FFM SDS and height adjusted DXA FFMI to predict 4C FFMI SDS.

Graphs are on following pages.

Figure 1a. Bland-Altman analyses of agreement between the 4-component model and dual-energy X-ray absorptiometry, skinfold thicknesses and body mass index in the assessment of fat mass and fat mass index.

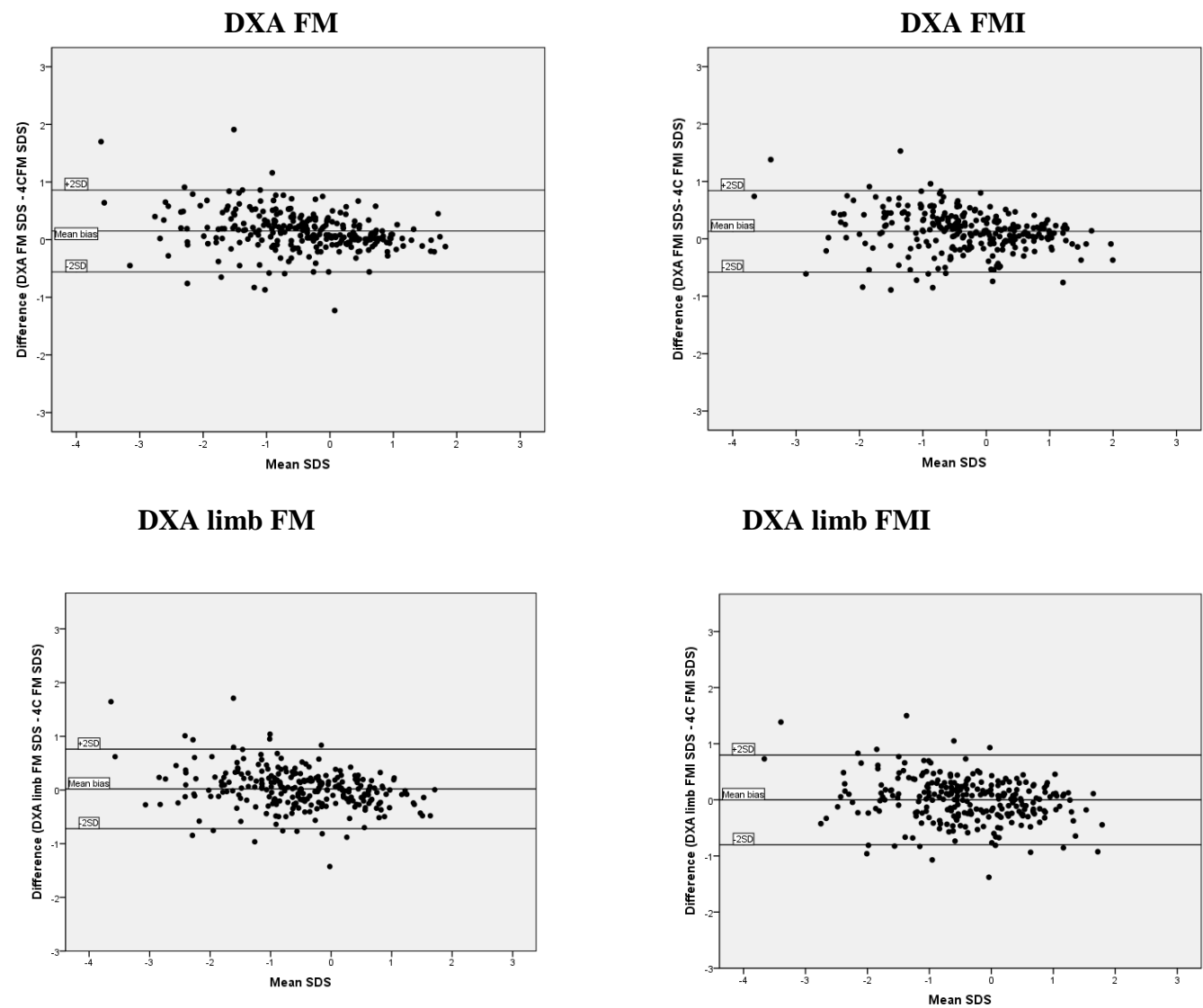


Figure 1b. Bland-Altman analyses of agreement between the 4-component model and dual-energy X-ray absorptiometry, skinfold thicknesses and body mass index in the assessment of fat mass and fat mass index.

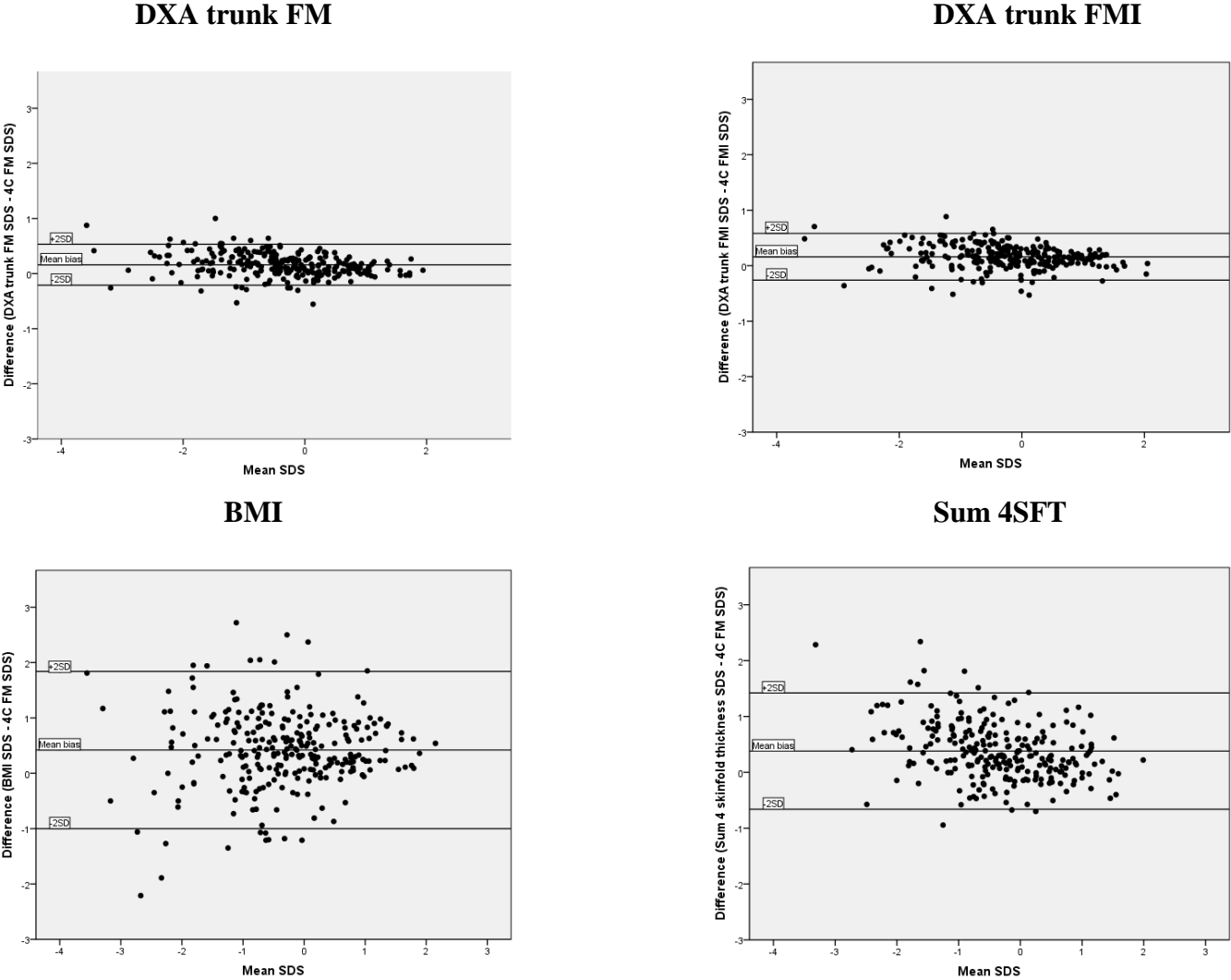


Figure 1c. Bland-Altman analyses of agreement between the 4-component model and dual-energy X-ray absorptiometry, skinfold thicknesses and body mass index in the assessment of fat mass and fat mass index.

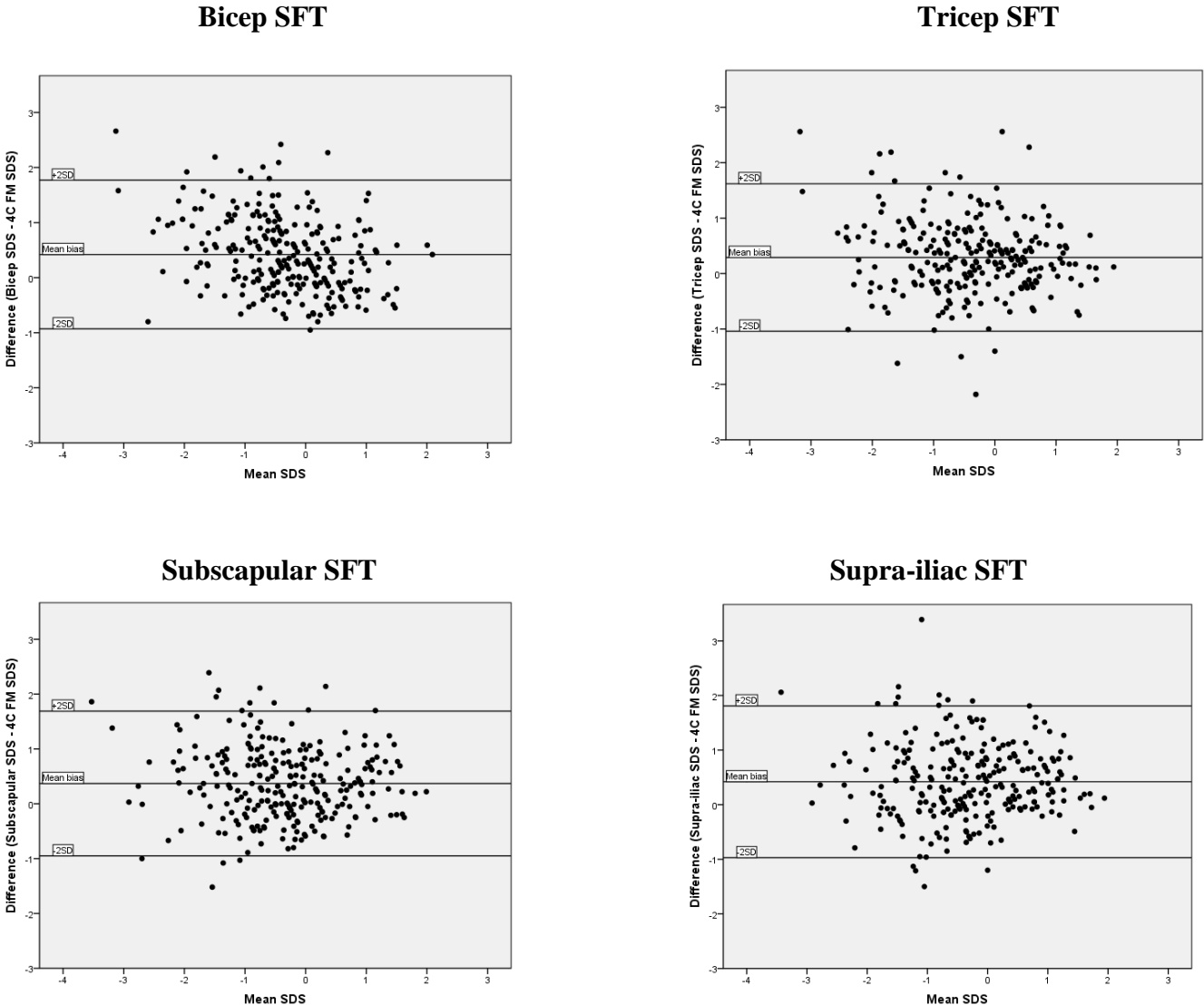


Figure 2a. Bland-Altman analyses of agreement between the 4-component model and dual-energy X-ray absorptiometry, total body water and bio-electrical impedance in the assessment of fat-free mass and fat-free mass index.

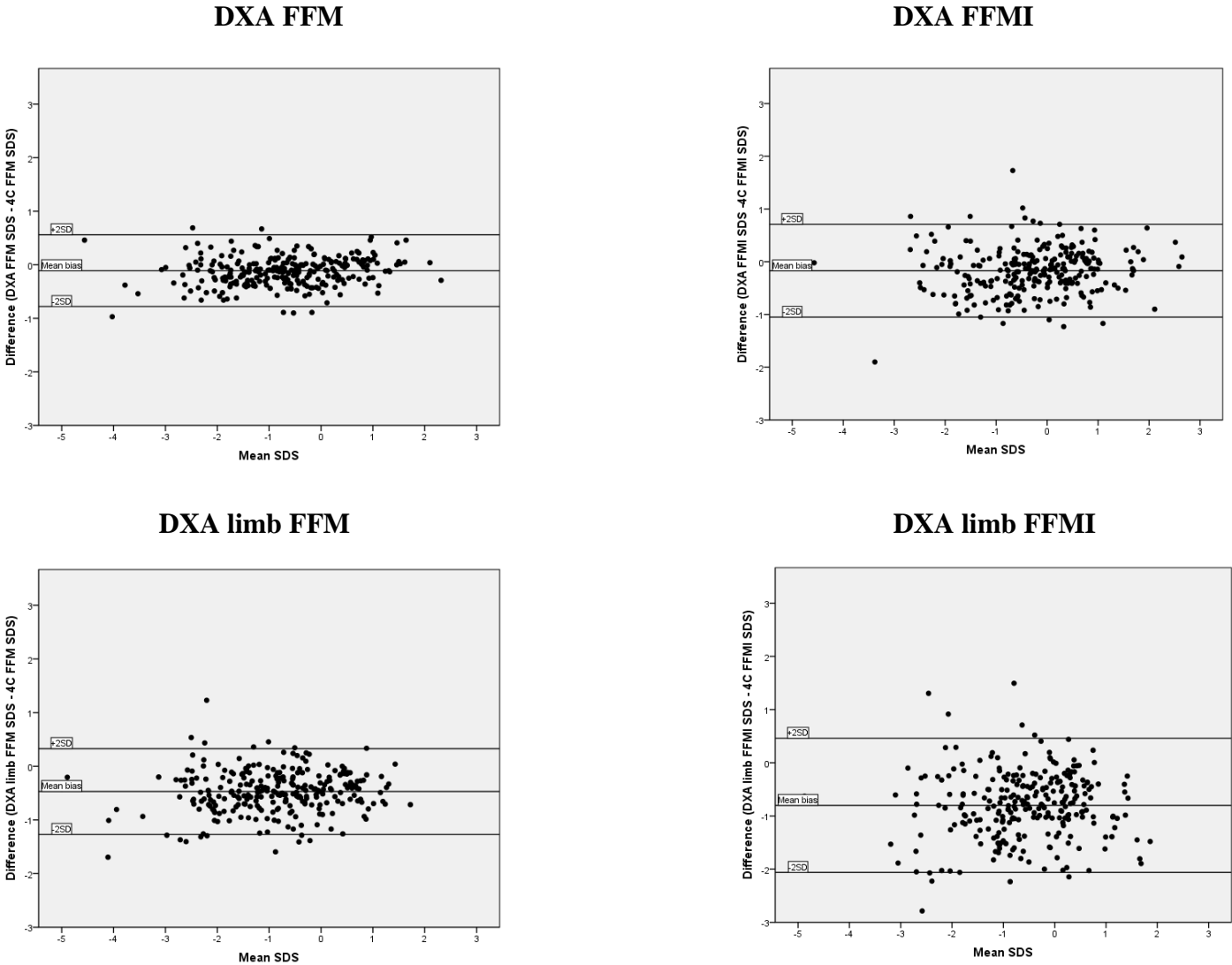
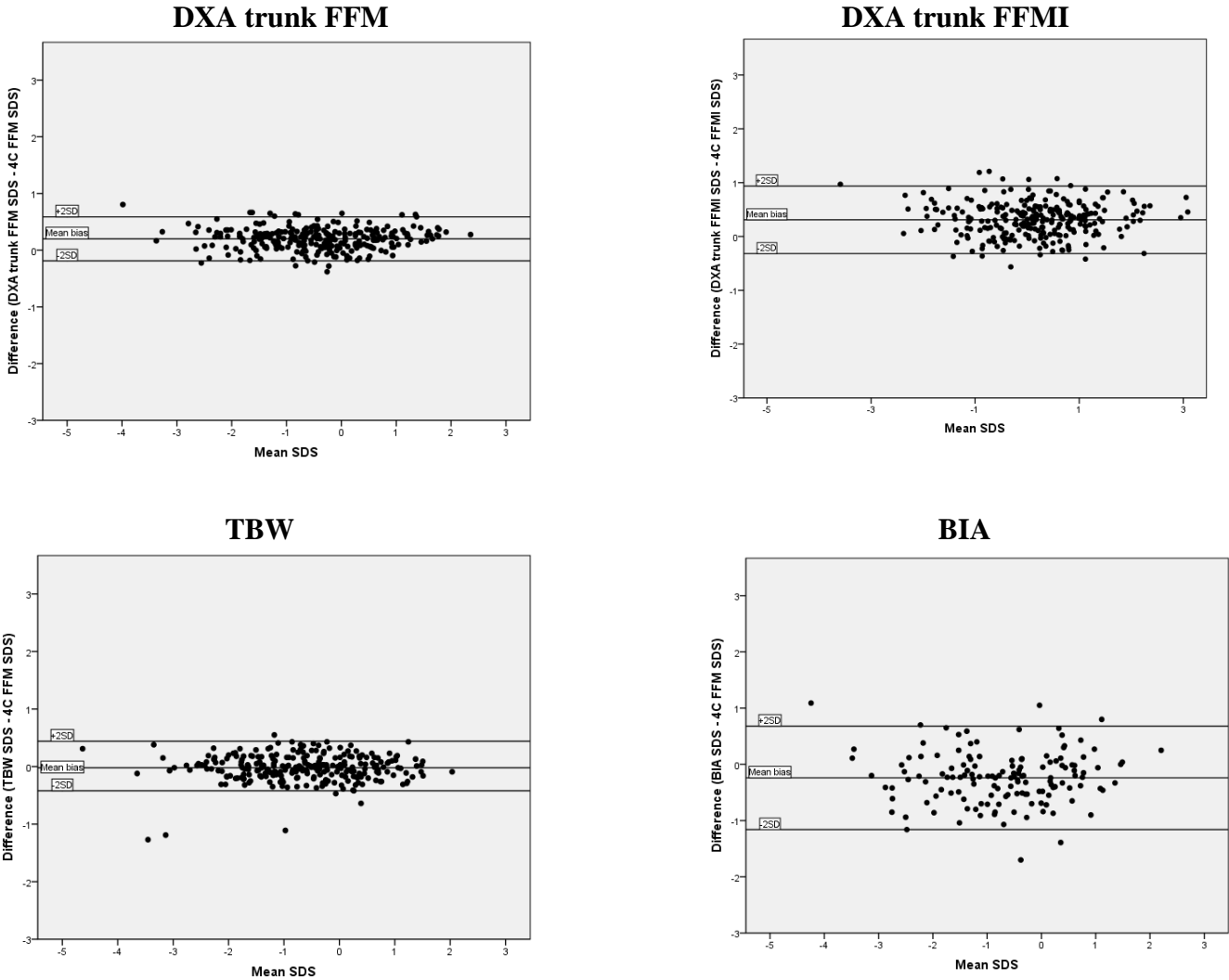


Figure 2a. Bland-Altman analyses of agreement between the 4-component model and dual-energy X-ray absorptiometry, total body water and bio-electrical impedance in the assessment of fat-free mass and fat-free mass index.



Appendix 13. Bland Altman correlations, unadjusted and adjusted for age, and age and sex

	<i>R</i> unadjusted	<i>P</i>	<i>R</i> adjusted for age	<i>P</i>	<i>R</i> Adjusted for age and sex	<i>P</i> ²
SDS						
<i>Measure of 'fatness'</i>						
DXA fat mass	-0.279	P<0.001	-0.281	P<0.001	-0.288	P<0.001
DXA fat mass index	-0.251	P<0.001	-0.251	P<0.001	-0.232	P<0.001
DXA limb fat mass	-0.294	P<0.001	-0.294	P<0.001	-0.303	P<0.001
DXA limb fat mass index	-0.233	P<0.001	-0.235	P<0.001	-0.220	P<0.001
DXA trunk fat mass	-0.282	P<0.001	-0.287	P<0.001	-0.292	P<0.001
DXA trunk fat mass index	-0.228	P<0.001	-0.230	P<0.001	-0.218	P<0.001
Bicep skinfold	-0.331	P<0.001	-0.334	P<0.001	-0.314	P<0.001
Tricep skinfold	-0.142	0.021	-0.145	0.018	-0.150	0.015
Subscapular skinfold	-0.072	0.245	-0.074	0.229	-0.049	0.424
Supra-iliac skinfold	0.001	0.982	0.001	0.982	0.009	0.886
Sum 4 skinfold	-0.350	P<0.001	-0.354	P<0.001	-0.339	P<0.001
BMI	0.049	0.429	0.039	0.531	0.042	0.492
<i>Measure of 'leanness'</i>						
DXA fat-free mass	0.209	0.001	0.217	P<0.001	0.214	P<0.001
DXA fat-free mass index	0.114	0.064	0.093	0.132	0.089	0.149
DXA limb fat-free mass	0.096	0.121	0.098	0.112	0.072	0.244
DXA limb fat-free mass index	0.049	0.424	0.048	0.435	0.041	0.507
DXA trunk fat-free mass	0.004	0.947	0.003	0.966	0.017	0.788
DXA trunk fat-free mass index	-0.002	0.977	-0.036	0.559	-0.022	0.723
Total body water	0.014	0.819	0.025	0.681	0.035	0.577
Bio-electrical impedance	0.003	0.975	0.019	0.826	0.004	0.967

² Pearson's correlation between the bias in individuals and the mean values (unadjusted and adjusted for age and for age and sex).

Appendix 14. Characteristics of children used and not used in generation of a regression equation to correct for bias in bio-electrical impedance standard deviation scores.

	N	Mean age y	SD	Range y	Males	Females
Included in the regression model	61	12.6	2.8	7.0-17.0	29	32
Excluded from the equation model (longitudinal subjects)	81	10.7	2.4	6.7-16.6	36	45
No bio-electrical impedance data	124	12.2	2.5	6.9-16.5	59	65

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Evaluation of Lunar Prodigy dual-energy X-ray absorptiometry for assessing body composition in healthy persons and patients by comparison with the criterion 4-component model¹⁻³

Jane E Williams, Jonathan CK Wells, Catherine M Wilson, Dalia Haroun, Alan Lucas, and Mary S Fewtrell

ABSTRACT

Background: Dual-energy X-ray absorptiometry (DXA) is widely used to assess body composition in research and clinical practice. Several studies have evaluated its accuracy in healthy persons; however, little attention has been directed to the same issue in patients.

Objective: The objective was to compare the accuracy of the Lunar Prodigy DXA for body-composition analysis with that of the reference 4-component (4C) model in healthy subjects and in patients with 1 of 3 disease states.

Design: A total of 215 subjects aged 5.0–21.3 y ($n = 122$ healthy nonobese subjects, $n = 55$ obese patients, $n = 26$ cystic fibrosis patients, and $n = 12$ patients with glycogen storage disease). Fat mass (FM), fat-free mass (FFM), and weight were measured by DXA and the 4C model.

Results: The accuracy of DXA-measured body-composition outcomes differed significantly between groups. Factors independently predicting bias in weight, FM, FFM, and percentage body fat in multivariate models included age, sex, size, and disease state. Biases in FFM were not mirrored by equivalent opposite biases in FM because of confounding biases in weight.

Conclusions: The bias of DXA varies according to the sex, size, fatness, and disease state of the subjects, which indicates that DXA is unreliable for patient case-control studies and for longitudinal studies of persons who undergo significant changes in nutritional status between measurements. A single correction factor cannot adjust for inconsistent biases. *Am J Clin Nutr* 2006;83:1047–54.

KEY WORDS Body composition, fat mass, fat-free mass, dual-energy X-ray absorptiometry, DXA, obesity, clinical practice

INTRODUCTION

Assessment of body composition is increasingly used to direct the clinical management of patients. First, an abnormal body composition (eg, high or low amounts of body fat) is often a major symptom of diseases such as obesity, with changes in body components reflecting the relative success of treatment. Second, the association of body composition with the risk of diseases, such as coronary heart disease, broadens its clinical significance. Third, body composition can also be used as the basis of requirements for fluids, nutrition, and dosages of drugs and dialysis. Despite increasing awareness of the value of such information, its measurement in routine practice has remained constrained by the lack of appropriate technology.

Dual-energy X-ray absorptiometry (DXA), first developed for assessment of bone mass, provides information on total fat mass (FM) and fat-free mass (FFM) and their distribution in the trunk and upper and lower limbs (1). Over the past decade, DXA has been increasingly used to assess body composition in research and clinical practice, including applications to direct treatment (2–4). Its rapid uptake can be attributed to its ease of use, availability, and low radiation exposure. However, although the precision of the technique for body-composition outcomes is well-established, insufficient attention has been paid to accuracy. Many validation studies have used as the reference method a technique that itself has unknown accuracy, thereby limiting confidence in the findings.

In the absence of chemical analysis of body composition, the ideal reference method is a multicomponent model of body composition, which minimizes the need for theoretical assumptions of biological constancy in tissues (5). A recent study evaluated Hologic Inc (Waltham, MA) DXA instrumentation against the 4-component (4C) model for estimating FM in a group of girls and adolescent females. A large bias and large limits of agreement were found between the 2 methods that could not be attributed to age, ethnicity, or fatness, but that could cause a person's FM to be under- or overestimated by 28% (6). These authors proposed that the bias could be addressed by a correction factor.

The latter study highlights important issues; however, further work is still required. First, the results may not apply to other manufacturer's instrumentation, because instruments differ in the way in which tissue masses are quantified. Second, many clinical applications involve extremes of body size and composition; however, the validity of DXA over a wide range of body sizes and health states has yet to be investigated. The aim of this study was to evaluate the level of agreement between DXA and the 4C model reference method when estimating FM, FFM, and

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weight in a diverse group of healthy and unhealthy adults and children to determine whether biases are consistent between these groups.

SUBJECTS AND METHODS

Subjects

A total of 215 subjects aged 5.0–21.3 y were recruited into studies of body composition in healthy subjects, adults born preterm, and patient groups from Great Ormond Street Children's Hospital and the National Hospital for Neurology and Neurosurgery. The sample consisted of nonobese adults ($n = 70$), obese adults ($n = 16$), healthy nonobese children ($n = 52$), obese children ($n = 39$), children with cystic fibrosis (CF; $n = 26$), and children with glycogen storage disease (GSD; $n = 12$). Obesity was defined as a body mass index (BMI; in kg/m^2) above the 95th percentile according to UK 1990 reference data (7). Two obese boys did not complete the protocol because it was not possible to scan their whole body because of the inadequate size of the scanning area. A separate analysis of data from obese men was not possible because of small sample sizes ($n = 2$). Measurements were conducted over a 90-min visit to the study center at Great Ormond Street Children's Hospital after a light meal. Ethical permission was obtained from the ethical committee of the Institute of Child Health and The National Hospital. Written consent was obtained from adults and parents. Written assent was obtained from children aged ≥ 11 y, and verbal assent was obtained from children aged < 11 y.

Dual-energy X-ray absorptiometry

Bone mineral content (BMC), FM, and FFM were determined by using a Lunar Prodigy whole-body scanner (GE Medical Systems, Madison, WI) in conjunction with Encore 2002 software. The instrument automatically alters scan depth depending on the thickness of the subject, as estimated from age, height, and weight. All scans were performed while the subjects were wearing light indoor clothing and no removable metal objects. The typical scan time was 5 min, depending on height. The radiation exposure per whole-body scan is estimated to be $2 \mu\text{Sv}$, which is lower than the daily background level. All scans were performed by one operator (CMW). The precision of soft tissue analysis for a Lunar DPX-L instrument (regarded by the manufacturers to be similar to the Lunar Prodigy), established by repeat measurements of humans on 4 successive days, has been reported as 1% for FFM and 2% for FM (8).

Body volume

Body volume (BV) was measured by using Bod Pod Instrumentation (Life Measurement Instruments, Concord, CA) according to the manufacturer's instructions as previously described (9). Measurements were made while the subjects wore a close-fitting swimming costume and hat. The raw volume values that appear transiently on the screen were recorded, and an adjustment for thoracic gas volume and surface area artifact was made to obtain actual BV as described previously (10). To improve precision, the procedure was repeated until 2 values for raw density of within $0.007 \text{ kg}/\text{L}$ were obtained (11). When it was not possible to achieve 2 such measurements, because of breathing irregularities in 3 of the children with CF, the mean of all raw volume values was used after values $\pm 2 \text{ SD}$ were discarded. All

measurements were made by 1 of 3 operators (JEW, CMW, or DH).

Anthropometric measurements

Body weight was measured as an integral stage of the Bod Pod procedure to within 0.01 kg . Accuracy was confirmed by the use of 2 solid weights of known mass. Height was measured to within 0.1 cm with a wall-mounted digital display stadiometer (Holtain, Dyfed, United Kingdom). BMI was calculated as weight (kg) divided by the square of height (m). Data on weight, height, and BMI were converted to SD scores (SDS) with the use of UK 1990 reference data (7, 12).

Deuterium dilution

Total body water (TBW) was assessed by deuterium (^2H -labeled water) dilution with the use of a dose equivalent to $0.05 \text{ g } ^2\text{H}_2\text{O}/\text{kg}$ body weight. Doses were made up with water to $\approx 100 \text{ mL}$ for young children and to 150 mL for older children and adults. Saliva samples were taken before the dose was administered and either 4 (for persons of normal body fatness) or 5 (for obese subjects) h after the dose was administered. Absorbent salivettes (Sarstedt, Rommelsdorf, Germany) were used to collect the saliva ≥ 30 min after the last ingestion of food or drink.

Deuterium samples were analyzed by Iso-Analytical Ltd (Sandbach, United Kingdom) by using the equilibration method of Scrimgeour et al (13). Briefly, 0.3 mL liquid, along with a vial of 5% platinum on alumina powder (Sigma-Aldrich, Poole, United Kingdom), was placed in a septum sealed container (Labco, High Wycombe, United Kingdom) and flushed for 2 min with hydrogen. Low-enrichment and high-enrichment standard waters were similarly prepared to normalize data against SMOW-SLAP (Standard Mean Ocean Water/Standard Light Arctic Precipitation) standards. Samples were equilibrated at room temperature for 3 d before analysis. The head spaces in the containers were then analyzed for deuterium enrichment with a continuous-flow isotope ratio mass spectrometer (Geo20-20; Europa Scientific, Crewe, United Kingdom). The accuracy of the analyses was checked by measuring an intermediate water standard within each batch of samples. All samples were prepared and analyzed in duplicate. The mean SD of deuterium analyses by the equilibration technique in the laboratory is $< 2.5\%$.

^2H dilution space was assumed to overestimate TBW by a factor of 1.044 (14), and a correction was made for fluid intake during the equilibrium period to derive actual body water.

Four-component model

The 4C model uses values of BMC, body weight (BW), BV, and TBW to derive values for mineral, water, fat, and protein as described previously (5). Assumed densities of the 4 components were accounted for when calculating fat mass from the measurements.

$$\text{FM (kg)} = [(2.747 \times \text{BV}) - (0.710 \times \text{TBW})] + [(1.460 \times \text{BMC}) - (2.050 \times \text{BW})] \quad (1)$$

FFM was calculated as the difference between weight and FM. We previously propagated error for FFM and FM on the basis of precision of the raw measurements and obtained values of 0.5 kg FM or FFM in children (15) and adults (5).



TABLE 1
Characteristics of the adults¹

	Nonobese men (n = 26)	Nonobese women (n = 44)	Obese women (n = 14)
Age (y)	20.4 ± 0.45	20.3 ± 0.40	20.4 ± 0.41
Weight SDS	-0.38 ± 1.13	-0.19 ± 1.17	1.90 ± 0.72 ^{2,3}
Height SDS	-0.27 ± 0.81	-0.17 ± 1.28	-1.01 ± 0.79 ^{2,4}
BMI SDS	-0.27 ± 1.14	-0.13 ± 0.96	2.35 ± 0.54 ^{2,3}

¹ All values are $\bar{x} \pm$ SD. SDS, SD score relative to the 1990 UK reference data (7, 12).

² Significantly different from zero, $P < 0.001$ (paired t test).

^{3,4} Significantly different from nonobese women (independent samples t test): ³ $P < 0.001$, ⁴ $P < 0.05$.

Statistical analysis

Comparisons for anthropometric and body-composition variables between groups were made by using analysis of variance (ANOVA). Data for males and females were pooled, unless a significant interaction between group and sex was present. Pairwise post hoc comparisons between groups were made where appropriate by using Tukey's test.

The method of Bland and Altman (16) was used to assess agreement between techniques. The mean difference between techniques (bias) and the ± 2 SDs of the difference between techniques (limits of agreement) were calculated. The bias was then tested for significance from zero by using a paired t test. The correlation between the bias and the mean of the measured values was also determined. To express the bias as a percentage of the mean, the bias of the individual natural log value multiplied by 100 was calculated (17, 18). We used analysis of covariance (ANCOVA) to examine factors predicting bias in FM, FFM, weight, and percentage body fat, including age, sex, disease state, and either BMI SDS, FM, or percentage body fat as independent variables. All analyses were performed by using the Statistical Package for Social Sciences (version 11.0; SPSS Inc, Chicago, IL), and a P value < 0.05 was considered significant.

TABLE 2
Characteristics of the children¹

	Group			
	Nonobese (n = 30 M, 22 F)	Obese (n = 11 M, 26 F)	CF (n = 12 M, 14 F)	GSD (n = 8 M, 4 F)
Age (y) ²				
Boys	10.3 ± 3.82	12.6 ± 2.66	9.47 ± 0.67	9.87 ± 2.90
Girls ³	10.2 ± 3.28	10.9 ± 2.48	10.2 ± 1.13	14.5 ± 2.30
BMI SDS ^{2,4}				
Boys	0.22 ± 0.89	2.77 ± 0.68 ⁵	0.44 ± 1.10	1.70 ± 1.26 ⁶
Girls	0.34 ± 0.57	2.77 ± 0.73 ⁵	-0.68 ± 1.47	0.77 ± 0.85
Weight SDS ^{4,7}	0.21 ± 0.87	2.68 ± 0.93	-0.42 ± 1.27	0.64 ± 1.28
Height SDS ^{4,7}	0.03 ± 0.91	0.81 ± 1.01 ⁵	-0.58 ± 1.05 ⁶	-0.75 ± 1.03 ⁸

¹ All values are $\bar{x} \pm$ SD. CF, cystic fibrosis; GSD, glycogen storage disease; SDS, SD score relative to the 1990 UK reference data (7, 12).

² Significant interaction between sex and group ($P < 0.05$) in 2-factor ANOVA; therefore, the sexes were analyzed separately.

³ ANOVA for condition, $P = 0.03$.

⁴ ANOVA for condition, $P < 0.001$.

^{5,6,8} Significantly different from zero (paired t test): ⁵ $P < 0.001$, ⁶ $P < 0.01$, ⁸ $P < 0.05$.

⁷ No significant interaction between sex and group ($P > 0.05$) in 2-factor ANOVA; therefore, the sexes were analyzed together.

RESULTS

Subject characteristics and body composition

The population encompassed a wide range of body sizes and nutritional status. The characteristics of the adults ($n = 84$) are shown in **Table 1**. The young adults (19–22 y) of normal weight were representative of the UK population in height and weight. There were no significant differences in age and weight, height, and BMI SDS between the sexes in the nonobese group. The obese women were significantly shorter and, by definition, heavier than the UK reference data.

Characteristics of the children ($n = 127$) are shown in **Table 2**. The subjects were deliberately chosen to represent a range of body sizes and shapes and, as expected, ANOVA showed significant differences in weight, height, and BMI SDS between groups. Compared with the UK reference data, the nonobese boys were representative of the general population in terms of height and weight SDS, but the nonobese girls were significantly heavier than expected ($P < 0.05$). The obese girls were significantly taller ($P < 0.001$), whereas the patient groups showed a wide range of body sizes: the children with CF were significantly shorter and the children with GSD were significantly shorter and heavier.

The body-composition data for adults are shown in **Table 3**. ANOVA showed significant differences between groups for all variables, and post hoc testing indicated that these significant differences were present between all groups for all variables except the BMC and FFM of obese and nonobese women and the density of FFM in the nonobese men and women.

Data for the children's body composition are given in **Table 4**. ANOVA showed significant differences between groups for all variables, except for the density of FFM ($P = 0.06$). Mean percentage fat varied widely between groups, averaging 42% in obese girls and 19% in nonobese boys. FFM also varied widely between groups. Hydration was significantly higher (1.6%; $P < 0.01$) in the obese children than in the nonobese children.

TABLE 3Body composition of the adults¹

	Nonobese men (n = 26)	Nonobese women (n = 44)	Obese women (n = 14)
BW (kg)	66.9 ± 8.83	57.3 ± 9.14	78.1 ± 9.52
BV (L)	63.0 ± 8.84	55.6 ± 9.32	78.3 ± 9.97
TBW (L)	40.9 ± 4.34	29.9 ± 3.99	33.5 ± 3.56
BMC by DXA (kg)	2.88 ± 0.45	2.31 ± 0.38	2.38 ± 0.25
DXA body composition			
FM (kg)	12.1 ± 5.80	18.6 ± 6.46	36.4 ± 6.49
FFM (kg)	55.8 ± 5.72	38.7 ± 5.10	41.3 ± 4.41
Percentage fat (%)	17.3 ± 6.75	32.1 ± 6.76	46.6 ± 3.59
Weight (kg)	67.9 ± 8.94	57.3 ± 9.14	77.6 ± 9.58
4-Component model			
FM (kg)	10.8 ± 5.27	17.4 ± 5.81	34.8 ± 6.55
FFM (kg)	56.2 ± 5.94	39.9 ± 5.37	43.3 ± 4.55
Percentage fat (%)	15.6 ± 6.26	29.9 ± 6.05	44.4 ± 3.9
H _{FFM} (%)	72.8 ± 1.39	74.7 ± 1.60	77.2 ± 1.07
D _{FFM} (kg/L)	1.101 ± 0.005	1.100 ± 0.006	1.091 ± 0.004

¹ All values are $\bar{x} \pm \text{SD}$. BW, body weight; BV, body volume; TBW, total body water; BMC, bone mineral content; DXA, dual-energy X-ray absorptiometry; FM, fat mass; FFM, fat-free mass; H_{FFM}, hydration of FFM; D_{FFM}, density of FFM. Significant differences between groups for all variables ($P < 0.001$) by ANOVA and for all variables ($P \leq 0.05$) except for BMC and FFM between nonobese and obese women and for D_{FFM} between nonobese men and women by post hoc Tukey's test.

Comparison between DXA and the 4C model

Results of the Bland-Altman analyses are given in **Table 5**. These results show the variable bias in FM, FFM, body weight, and percentage fat in the different subject groups. DXA-measured weight was significantly underestimated in obese women and children, except the nonobese and obese boys, and was significantly overestimated in nonobese men. FM was significantly overestimated in all adults and obese boys and was significantly underestimated in nonobese boys. However, the bias in FFM did not mirror that in FM, as might have been

expected. This finding was accounted for by variable bias in weight between groups. Correlation analyses showed that the magnitude of the bias was related to the magnitude of the variable in several categories (Table 5). DXA measurement significantly underestimated percentage fat in nonobese boys, showed no significant bias in nonobese girls and children with CF or GSD, and overestimated it in obese children and all 3 categories of adults.

Factors predicting bias between DXA and the 4C model, by ANCOVA, are shown in **Table 6**. FM bias was significantly positively associated with both age and BMI SDS. FFM bias was

TABLE 4Body composition of the children¹

	Group			
	Nonobese (n = 30 M, 22 F)	Obese (n = 11 M, 26 F)	CF (n = 12 M, 14 F)	GSD (n = 8 M, 4 F)
BW (kg) ²	36.1 ± 14.0 ³	67.4 ± 21.8	30.1 ± 6.27 ^{3,4}	42.5 ± 12.3 ³
BV (L) ²	34.6 ± 13.3 ³	67.4 ± 22.2	28.9 ± 6.46 ³	41.7 ± 12.2 ³
TBW (L) ²	21.0 ± 8.29 ³	29.7 ± 8.60	17.9 ± 2.51 ³	21.9 ± 5.68 ³
BMC by DXA (kg) ²	1.35 ± 0.64 ³	1.90 ± 0.59	1.07 ± 0.16 ³	1.29 ± 0.51 ³
DXA body composition				
FM (kg) ²	7.76 ± 4.61 ³	29.2 ± 12.0	6.45 ± 3.60 ^{3,4}	13.7 ± 6.30 ³
FFM (kg) ²	28.3 ± 11.4 ³	37.9 ± 11.4	23.4 ± 3.20 ³	28.7 ± 7.21 ³
Percentage fat (%) ²	21.5 ± 7.84 ^{3,4}	42.6 ± 6.40	20.3 ± 7.33 ^{3,4}	31.0 ± 8.13 ³
DXA weight (kg) ²	36.0 ± 14.1 ³	67.1 ± 21.9	29.9 ± 6.36 ³	42.3 ± 13.5 ³
4-component model				
FM (kg) ²	8.05 ± 4.36 ³	28.6 ± 12.2	6.42 ± 3.74 ^{3,4}	13.7 ± 5.59 ³
FFM (kg) ²	28.1 ± 11.4 ³	38.8 ± 11.5	23.7 ± 3.16 ³	28.8 ± 7.80 ³
Percentage fat (%) ²	22.0 ± 7.02 ^{3,4}	41.4 ± 7.25	20.0 ± 7.54 ^{3,4}	31.5 ± 6.70 ³
H _{FFM} (%) ²	75.1 ± 1.99 ³	76.4 ± 1.73	75.3 ± 2.37	76.1 ± 1.98
D _{FFM} (kg/L)	1.091 ± 0.008	1.089 ± 0.006	1.089 ± 0.007	1.086 ± 0.009

¹ All values are $\bar{x} \pm \text{SD}$. CF, cystic fibrosis; GSD, glycogen storage disease; BW, body weight; BV, body volume; TBW, total body water; BMC, bone mineral content; DXA, dual-energy X-ray absorptiometry; FM, fat mass; FFM, fat-free mass; H_{FFM}, hydration of FFM; D_{FFM}, density of FFM.

² Significant difference across groups, $P < 0.001$ (ANOVA).

³ Significantly different from the obese group, $P \leq 0.006$ (post hoc Tukey's test).

⁴ Significantly different from the GSD group, $P \leq 0.006$ (post hoc Tukey's test).



TABLE 5

Bland-Altman analysis of mean bias in fat mass (FM), fat-free mass (FFM), weight, and percentage fat measured by dual-energy X-ray absorptiometry (DXA) compared with the 4-component model¹

	Absolute bias ²	Absolute 95% limits of agreement	Bias as a percentage of the mean ³	95% limits of agreement as a percentage of the mean	P for the bias from zero ⁴	r ⁵	P for the correlation
			%	%			
FM							
Adults							
Nonobese men (n = 26)	1.35	±2.82	12.4	±25.3	< 0.001	0.38	NS
Nonobese women (n = 44)	1.21	±2.29	6.19	±14.8	< 0.001	0.58	< 0.01
Obese women (n = 14)	1.58	±3.50	4.60	±11.0	< 0.01	-0.04	NS
Children							
Nonobese boys (n = 30)	-0.52	±1.17	-10.9	±23.8	< 0.001	0.10	NS
Nonobese girls (n = 22)	0.01	±1.24	9.72	±10.6	NS	0.46	< 0.05
Obese boys (n = 11)	0.93	±1.86	3.52	±7.06	< 0.01	-0.08	NS
Obese girls (n = 17)	0.46	±2.42	2.26	±9.39	NS	-0.24	NS
Boys with CF (n = 12)	-0.14	±1.83	0.38	±29.9	NS	-0.53	NS
Girls with CF (n = 14)	0.18	±1.42	0.77	±26.6	NS	0.48	NS
Boys and girls with GSD (n = 12)	-0.06	±2.28	-3.67	±22.6	NS	0.63	< 0.05
FFM							
Adults							
Nonobese men (n = 26)	-0.37	±2.71	-0.62	±4.88	NS	-0.18	NS
Nonobese women (n = 44)	-1.19	±2.38	-2.96	±6.06	< 0.01	-0.23	NS
Obese women (n = 14)	-1.97	±3.39	-4.68	±8.45	< 0.001	-0.08	NS
Children							
Nonobese boys (n = 30)	0.56	±1.51	2.06	±4.67	< 0.001	0.19	NS
Nonobese girls (n = 22)	-0.34	±1.38	-0.99	±4.99	< 0.05	-0.56	< 0.01
Obese boys (n = 11)	-1.02	±1.99	-2.49	±4.38	< 0.01	-0.03	NS
Obese girls (n = 17)	-0.80	±2.49	-2.25	±6.12	< 0.01	-0.05	NS
Boys with CF (n = 12)	-0.04	±1.76	-0.28	±7.13	NS	0.30	NS
Girls with CF (n = 14)	-0.53	±1.20	-2.20	±5.14	< 0.01	-0.40	NS
Boys and girls with GSD (n = 12)	-0.10	±2.28	0.13	±7.71	NS	-0.51	NS
Weight							
Adults							
Nonobese men (n = 26)	0.99	±0.92	1.46	±1.33	< 0.001	0.23	NS
Nonobese women (n = 44)	0.02	±0.50	0.04	±0.88	NS	-0.02	NS
Obese women (n = 14)	-0.39	±0.78	-0.52	±1.01	< 0.01	0.16	NS
Children							
Nonobese boys (n = 30)	0.04	±0.70	-0.64	±1.67	NS	0.61	< 0.01
Nonobese girls (n = 22)	-0.32	±0.50	-0.92	±1.22	< 0.001	-0.47	< 0.05
Obese boys (n = 11)	-0.09	±0.89	-0.19	±1.10	NS	0.37	NS
Obese girls (n = 17)	-0.33	±0.68	-0.63	±1.31	< 0.001	0.28	NS
Boys with CF (n = 12)	-0.18	±0.51	-0.67	±2.43	< 0.05	0.21	NS
Girls with CF (n = 14)	-0.35	±0.70	-1.28	±2.48	< 0.01	0.35	NS
Boys and girls with GSD (n = 12)	-0.17	±0.41	-0.44	±1.16	< 0.05	0.06	NS
Percentage fat (%)							
Adults							
Nonobese men (n = 26)	1.69	±3.81	—	—	< 0.001	0.26	NS
Nonobese women (n = 44)	1.98	±3.94	—	—	< 0.001	0.25	NS
Obese women (n = 14)	2.28	±4.72	—	—	< 0.05	-0.16	NS
Children							
Nonobese boys (n = 30)	-1.74	±3.52	—	—	< 0.001	0.09	NS
Nonobese girls (n = 22)	-0.03	±3.51	—	—	NS	0.44	< 0.05
Obese boys (n = 11)	1.41	±2.59	—	—	< 0.01	-0.54	NS
Obese girls (n = 17)	1.03	±3.50	—	—	< 0.01	-0.52	< 0.01
Boys with CF (n = 12)	-0.18	±5.54	—	—	NS	-0.42	NS
Girls with CF (n = 14)	0.65	±4.55	—	—	NS	0.41	NS
Boys and girls with GSD (n = 12)	-0.53	±5.59	—	—	NS	0.52	NS

¹ CF, cystic fibrosis; GSD, glycogen storage disease. Differential bias by group is evaluated by using analysis of covariance in Table 6.

² The mean value measured by DXA minus the mean value measured with the 4-component model.

³ The bias of the natural log values multiplied by 100.

⁴ Paired *t* test.

⁵ Pearson's correlation between the bias in individuals and the mean values.

significantly associated with age, sex, BMI SDS, and CF, and there was a significant interaction between age and BMI SDS, which indicated that BMI SDS was associated with decreased

bias with increasing age. Weight bias was significantly associated with age, sex, and BMI SDS. The use of an alternative model, in which BMI SDS was replaced with FM or percentage



TABLE 6

Analysis of factors predicting for bias between dual-energy X-ray absorptiometry and the 4-component (4C) model by analysis of covariance¹

Factor	β Coefficient	<i>t</i>	<i>P</i>	Adjusted <i>r</i> ²
FM bias				
Age	0.14	8.85	< 0.001	0.32
BMI SDS	0.21	4.01	< 0.001	
FFM bias				
Age ²	-0.099	-6.18	< 0.001	0.29
Sex	0.74	4.8	< 0.001	
BMI SDS	-0.26	-4.87	< 0.001	
CF	0.51	2.02	0.05	
Weight bias				
Age ^{3,4}	0.041	7.05	< 0.001	0.45
Sex	0.55	9.76	< 0.001	
BMI SDS ⁵	-0.049	-2.5	0.013	
Percentage fat bias				
Age ⁶	0.25	8.78	< 0.001	0.34
Sex	-0.89	-3.2	0.002	
BMI SDS	0.43	4.49	< 0.001	
CF	-1.27	-2.79	0.006	

¹ For each bias we performed general linear models that included the following factors: age, sex (M = 0, F = 1), CF (healthy = 0, CF = 1), GSD (healthy = 0, GSD = 1), and BMI SDS, FM, or percentage fat. The significant factors (*P* < 0.05) for each model are shown. Interactions between significant factors for each model were then tested and are reported with the adjusted mean values for significant categorical variables as follows: FFM bias (male, -0.29; female, -1.03; healthy, -0.4; CF, -0.91); weight bias (male, 0.17; female, -0.37); percentage fat bias (male, 0.50; female, 1.39; healthy, 0.31; CF, 1.58). SDS, SD score relative to the 1990 UK reference data (7, 12); CF, cystic fibrosis; GSD, glycogen storage disease; FM, fat mass; FFM, fat-free mass.

² Significant interaction between age and BMI SDS (coefficient = -0.026, *P* = 0.021).

³ Significant interaction between age and BMI SDS (coefficient = -0.007, *P* = 0.04).

⁴ Significant interaction between sex and age (coefficient = 0.06, *P* < 0.001).

⁵ Similar results were also obtained if BMI SDS was replaced with FM (coefficient = -0.009, *P* < 0.001) or percentage fat (coefficient = -0.015, *P* < 0.001).

⁶ Significant interaction between age and sex (coefficient = 0.13, *P* = 0.03).

fat, showed that weight bias was associated with age, sex, and either FM or percentage fat. Bias in percentage fat was associated with age, sex, BMI SDS, and CF, and there was a significant

interaction between age and sex, which indicated that the effect of being female on percentage fat bias was greater with increasing age. When waist circumference (as an alternative proxy for depth of scan) was substituted for BMI SDS in the ANCOVA, the results for all 4 body-composition biases were similar to those for BMI SDS (data not shown).

Propagation of error analysis indicated that methodologic imprecision was equivalent to ≈ 0.6 kg of bias. Biases of ≤ 0.6 kg could be attributed to imprecision, but larger biases could be attributed to the combination of imprecision and inaccuracy. For biases > 1 kg, most of the bias could be attributed to the inaccuracy of one or other method.

DISCUSSION

DXA was designed for the measurement of bone mass and has been shown to be both accurate and precise when used for this purpose. However, this method is increasingly being used for the measurement of body composition. Our study was the first to examine the validity of DXA in groups differing in body size, fatness, and (due to the effects of disease states) the chemical composition of FFM. We showed that the bias of DXA varied according to body size, body fatness, sex, and disease state. These findings indicate that caution is necessary when DXA is used to compare patients with control subjects or to assess changes in body composition in persons whose relative weight changes significantly between measurements.

Many studies have investigated the accuracy of DXA. Animal carcass studies have shown systematic biases in younger age groups, which required a correction factor to be generated (19) and applied (20, 21). However, most studies in humans have used reference methods that may not have been accurate. Two-component techniques, such as hydrodensitometry, rely on assumed constant properties of FM and FFM. We showed previously that this is not the case in healthy adults (5) or children (15), and the issue is of even greater importance when measuring patients in whom body-composition variability, especially FFM composition, is most extreme.

Recently, several studies have assessed DXA in relation to the 4C model. These studies are summarized in **Table 7**, and they highlight 2 issues: 1) the bias varies according to several factors, including subject age and instrumentation, and 2) the vast majority of work has been conducted in healthy adults and children.

TABLE 7

Summary of studies that assessed dual-energy X-ray absorptiometry against a 4-component model

Reference	Date	Instrumentation	No. of subjects	Subjects	Percentage fat bias	Significance	Percentage fat limits of agreement
					%		
Prior et al (22)	1997	QDR1000W ¹	172	Adults	0.4	No	5.8
Roemmich et al (23)	1997	QDR2000 ¹	47	Children	1.88	Yes	8.3
Wells et al (15)	1999	QDR1000W ¹	30	Children	-0.2	No	6.5
Clasey et al (24)	1999	QDR2000 ¹	78	Adults	-0.9 to -4.5	Yes	7.8-10.5
Wong et al (6)	2002	QDR2000W ¹	141	9-17 y	-3.9	Yes	3.4
Fuller et al (5)	1992	DPX ²	28	Adults	-1.36	Yes	4.95
Gately et al (25)	2003	Lunar ²	30	Obese children	1.9	Yes	4.0
Van der Ploeg et al (26)	2003	DPX-L ²	152	Adults	-1.8	Yes	4.0
Sopher et al (27)	2004	DPX/DPX-L ²	411	Children	1.01	Yes	8.9

¹ Hologic Inc, Waltham, MA.

² GE Medical Systems, Madison, WI.

Demonstration of the validity of DXA in healthy subjects is not sufficient justification for its application in patients, and our findings are highly relevant to this issue.


Many factors may contribute to the differences between studies. First, DXA assumes a constant value for FFM hydration; however, this may not apply to all categories of subjects and, hence, may represent an inappropriate bias that introduces error (22, 28–32). One study, however, suggested that the magnitude of this bias is likely to be small (30). Second, subject size may influence bias through the effect of tissue depth (29), with increasing tissue depth associated with greater bias by DXA (33). Third, some DXA instruments have no algorithms specific to children. Fourth, accuracy may differ between pencil- and fan-beam DXA instruments (34). Fifth, fat distribution may influence accuracy, because pixels containing bone (approximately one-third of the total) extrapolate soft tissue composition from adjacent regions, which may have a fat content different from the region overlying the bone (29). Sixth, most of the head soft tissue composition is not calculated. Seventh, DXA instruments vary in the approach used to estimate the fat content of bone, which leads to generic differences between manufacturers. Finally, it is possible that 4C models differ according to whether BV is measured by underwater weighing or plethysmography, although most studies have shown good agreement between these methods (35). Note that our own findings apply only to Lunar Prodigy instrumentation.

The results of our study highlight variable bias in the measurement of FM, FFM, and weight by DXA according to several key characteristics of subjects. Clearly, variable bias between patients and healthy subjects presents difficulties for case-control studies. Bias in weight has particular relevance to longitudinal studies because it may confound the estimation of changes in body composition (36). The sex-difference in bias has implications for studies intending to derive body composition cutoffs for overweight and obesity (37, 38). The difference in bias between obese and nonobese persons indicates that DXA may be unsuitable for assessing changes in body composition during weight loss, as was reported in several studies (24, 39). However, body size and fatness did not fully account for the variability in bias between groups: children with GSD, though fatter than nonobese children, did not have biases similar to those of obese children. Because the bias was inconsistent, it would be difficult to derive a simple single correction factor, as has been proposed by others working on more homogenous samples (6).

The magnitude of mean biases found in our study was ≤ 2 kg of FFM and FM in groups, equivalent to $\leq 2\%$ fat. In individuals, the limits of agreement were ≤ 3 kg of FFM and FM in adults and ≤ 2 kg in children, equivalent to 4–6% fat depending on age and group. This range of bias is smaller than that reported by Wong et al (6), but remains a serious issue because it is potentially of the order of magnitude of difference that might be expected after treatment in an individual or between groups. Factors including size, age, sex, and disease state all showed an independent effect in ANCOVA, which suggests that qualitatively different factors contribute to DXA bias. Our data indicate that DXA has limitations for measurement of body composition in clinical practice, but our findings are also important in the context of research studies. The literature contains increasing numbers of studies using DXA to undertake clinical research intended to provide

evidence appropriate as the basis for clinical practice. Our findings challenge the validity of this approach and suggest that other approaches, such as multicomponent models, are preferable.

The main limitation of our study, which is common to all studies comparing DXA with the 4C model, was that DXA provides data for both measurements. The measurement of BMC is integral to DXA calculations of FFM and FM, and the same data are also used in the 4C model. However, we believe that our study is not adversely affected by this scenario. First, we calculated that BMC would need to be measured with $>30\%$ error to induce a 2% error in percentage fat. Thus, we think it highly unlikely that our finding of variable bias between category of subjects can be entirely attributed to an effect of BMC error on both methods. Second, we reran our Bland-Altman calculations using the 3-component model, which incorporates no data from DXA and is therefore fully independent, and obtained similar results. We chose the 4C model as the reference because only this method can address the variability in mineralization that occurs within and between groups. Although the results are limited to the groups being studied, it is sufficient to highlight that the accuracy with which DXA measures body composition varies depending on several factors.

In conclusion, our study suggests that caution is required in the application of this instrumentation in the measurement of body composition in medical research and clinical practice. Our findings may be particularly challenging for randomized controlled trials, in which differences in body composition at follow-up may induce inconsistent accuracy between 2 groups. We suggest that multicomponent models remain the best existing method for underpinning the evidence base for body-composition studies. 

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JCKW and MSF conceived the study. JEW, CMW, and DH measured the subjects and modeled the body-composition data. JEW, JCKW, and MSF conducted the statistical analyses. JEW wrote the first draft of the manuscript. All authors contributed to the revision of the manuscript. None of the authors had a conflict of interest.

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Pediatric reference data for lean tissue properties: density and hydration from age 5 to 20 y¹⁻³

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ABSTRACT

Background: Hydrometry and densitometry are widely used to assess pediatric body composition due to their ease of application. The accuracy of these techniques depends on the validity of age- and sex-specific constant values for lean tissue hydration or density. Empirical data on these constants, and their variability between individuals, are lacking.

Objectives: The objectives were to measure lean tissue hydration and density in a large sample of children and adolescents and to derive prediction equations.

Design: Body composition was measured in 533 healthy individuals (91% white) aged 4–23 y by using the 4-component model. Age- and sex-specific median values for hydration and density were obtained by using the LMS (lambda, mu, sigma) method. Regression analysis was used to generate prediction equations on the basis of age, sex, and body mass index SD score (BMI SDS). Values were compared with those in previously published predictions.

Results: Age-associated changes in density and hydration differed between the sexes. Compared with our empirical values, use of published values resulted in a mean bias of 2.1% fat ($P < 0.0001$). Age, sex, and BMI SDS were all significant predictors of lean tissue hydration and density. With adjustment for age and sex, hydration was higher, and density lower, in higher-BMI SDS individuals.

Conclusions: The chemical maturation of lean tissue is not a linear process and proceeds differently in males and females. Previously published reference values are inaccurate and induce clinically significant bias in percentage fat. New empirical reference values are provided for use in pediatric hydrometry and densitometry. Further research that extends to cover nonwhite ethnic groups is needed. *Am J Clin Nutr* 2010;91:610–8.

INTRODUCTION

The gold standard for body-composition assessment at the molecular level is cadaver dissection; hence, all in vivo measurements in humans are restricted to the measurement of specific physical parameters, such as body density, body water or potassium content, or X-ray attenuation. Raw data on such variables are converted to final body-composition values by using assumptions concerning the biological, chemical, or physical properties of specific body components. Two useful body-composition techniques, especially in children, are hydrometry [the measurement of total body water (TBW)] and densitometry. In hydrometry, TBW is converted to lean tissue (used here syn-

onymously with fat-free tissue) by using an assumed constant value for lean tissue hydration (1). The same correction is required with the use of most bioelectrical impedance equations, which usually predict TBW from the raw bioelectrical data (2). In densitometry, the proportion of fat in weight (% fat) is calculated on the basis of assumed constant densities of fat and lean tissues (3). Although these techniques are regularly used for pediatric body-composition assessment, their accuracy is dependent not only on accuracy of the raw data but also on the accuracy of the tissue property values assumed to be constant among individuals.

These issues are of particular importance when studying children due to the well-established process of chemical maturation that occurs before adulthood (4, 5). Whereas fat has relatively uniform physical properties throughout the life course (zero water content and a density of 0.9007 kg/L), lean tissue decreases in water content and increases in density during growth (5). At birth, lean tissue has been proposed to be ≈80% water, in contrast to the adult value of ≈73% (5). Lean tissue density likewise increases from ≈1.063 to ≈1.10 kg/L over the same period (5). For almost 3 decades, researchers have relied on 2 landmark publications for these tissue constants. In 1982 Fomon et al (5) published a “reference child,” providing such data for the period from birth to age 10 y. However, these data were based on actual measurements only in infancy and at age 9 or 10 y, with most values in between being extrapolated. In 1989 Lohman (6) published similar reference data for the entire pediatric age range, merging the data of Fomon et al with further empirical data based on simultaneous measurements of TBW, body density, and forearm bone mineral density in 292 individuals aged 8–30 y (7). Simulations for adolescents were also reported by Haschke (8).

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Further data on these tissue properties have been reported for narrow age ranges, and typically in small samples (7, 9–12), although more comprehensive data for the first 2 y of life are available (13). Currently, researchers cannot be confident of selecting appropriate age- and sex-specific values, and there is little understanding of other factors associated with individual variability in these tissue properties. For example, we previously reported an association between hydration and body mass index (BMI) SD score (BMI SDS) in children (14).

We have recently obtained data on body composition in a large, healthy sample of children and adolescents aged 4–23 y. Here, we report reference data for the hydration and density of lean tissue and develop prediction equations on the basis of age, sex, and BMI SDS.

SUBJECTS AND METHODS

A total of 565 normal, healthy children and adolescents aged 4–23 y were recruited by using flyers and newspaper advertisements in London and the southeast of England, starting in 2001. There were no exclusion criteria for BMI; hence, some individuals were categorized as overweight or obese, but they were not recruited directly from obesity weight-loss clinics and had no disease that might have adversely affected growth and development. The lower age limit of 4 y was chosen on the basis of our previous work, in that younger children are unlikely to satisfy the protocol for air-displacement plethysmography. Data collection was extended to young adults to cover the entire pediatric age range. Ethical approval was granted by the Ethical Committee of the University College London Institute of Child Health and Great Ormond Street Hospital. All individuals attended our body-composition investigation suite located at Great Ormond Street Hospital for a 2-h measurement session.

Weight and height were measured by using standard protocols. Body weight (BW) was measured in duplicate as part of the air-displacement plethysmography protocol (*see below*). Height was measured by using a wall-mounted stadiometer (Holtain, Dyfed, United Kingdom). BMI was calculated as weight (kg) divided by the square of height (m). Anthropometric data were converted to SDS format by using UK reference data (15, 16). Obesity was defined as a BMI >95th centile (SDS >1.64), and overweight was defined as a BMI >85th centile (SDS >1.04) (16). Pubertal development was assessed by Tanner staging by self-assessment on the basis of line drawings. Skinfold thickness measurements were performed in triplicate at the biceps, triceps, subscapular, and suprailiac sites, and the mean of the 3 values was used.

Measurements of TBW by deuterium dilution, bone mineral content (BMC) by dual-energy X-ray absorptiometry (DXA; Lunar Prodigy; GE Medical Systems, Madison, WI), and body volume (BV; in duplicate) by air-displacement plethysmography (Bodpod; Life Measurements, Concord, CA) were obtained as described previously (17). The deuterium dilution space was converted to TBW assuming the degree of overestimation attributable to proton exchange to be 1.044 (18). Lung volume was predicted rather than measured in the plethysmography measurements, because we have found a large proportion of children are unable to complete the lung volume measurement protocol. The 4-component (4C) model was used to calculate lean mass and

fat mass (FM), as described previously (10, 19), using the following equation:

$$FM = (2.747 \times BV) - (0.710 \times TBW) + (1.460 \times BMC) - (2.050 \times BW) \quad (1)$$

The precision of TBW in our laboratory is 1% (10). The precision of BV from duplicate measurements and BMC is 0.24 L and 1.1%, respectively (20). Hydration was calculated as $(TBW / \text{lean mass}) \times 100\%$. Density was calculated as $[(\text{mass of water} + \text{mass of mineral} + \text{mass of protein}) / (\text{volume of water} + \text{volume of mineral} + \text{volume of protein})]$ with the use of values for the density of water at 36°C, mineral, and protein of 0.99371, 3.0375, and 1.34 kg/L, respectively (17).

Sex-specific values by year of age were obtained for hydration and density by using Cole's LMS (lambda, mu, sigma) method (LMS Chart Maker; Medical Research Council, London, United Kingdom) (21). This statistical approach, widely used to construct reference data for traits that incorporate the effects of growth, provides 3 outputs: 1) a smoothed median (M or mu) curve, which represents how the outcome varies in relation to age; 2) the CV (S or sigma), which models the scatter of values around the mean and adjusts for any nonuniform dispersion; and 3) the skewness (L or lambda), which is addressed by using age-specific Box-Cox transformation (L) to achieve a normal distribution. Goodness-of-fit was assessed by comparing consecutive models and adding complexity only if a significant improvement to the fit (reduced deviance) was obtained. Because the precision of the M curve at any age depends on data points at younger and older ages, precision is lower at the extremes of the age range. We therefore fitted the data for all ages (4–23 y) and derived M values for the age range of 5–20 y.

Derived values for the density of lean tissue (D_L) were combined with an assumed constant density of fat (D_F : 0.9007 kg/L) to generate age-specific constants for application in the generic equation proposed by Siri (3), as undertaken previously by Lohman (6):

$$\% \text{ Fat} = (C1/BD - C2) \times 100 \quad (2)$$

where BD is body density, C1 is calculated as $(D_L \times D_F) / (D_L - D_F)$, and C2 is calculated as $D_F / (D_L - D_F)$ (6).

Multiple regression analysis was undertaken to derive predictive equations for hydration and density of lean tissue. The independent variables comprised age, age squared, sex, and BMI SDS. These values are readily obtainable in community or clinical studies. Our aim was to derive appropriate equations and to identify the proportion of variance accounted for by the predictors. We also compared the utility of sex-specific equations with sex-combined equations. The association of BMI with hydration and density was assessed by using partial correlations adjusted for age and sex.

Residual values for hydration and density were calculated as the measured value minus the average (LMS-derived) value for the appropriate year age group. Correlation analysis was then used to investigate possible associations between the residual values and BMI SDS or skinfold thicknesses. The effect of obesity (categorized as yes/no) on hydration and density after adjustment for age, sex, and ethnicity was also tested for.

The method of Bland and Altman was used to compare our empirical values of % fat with those that were calculated by using

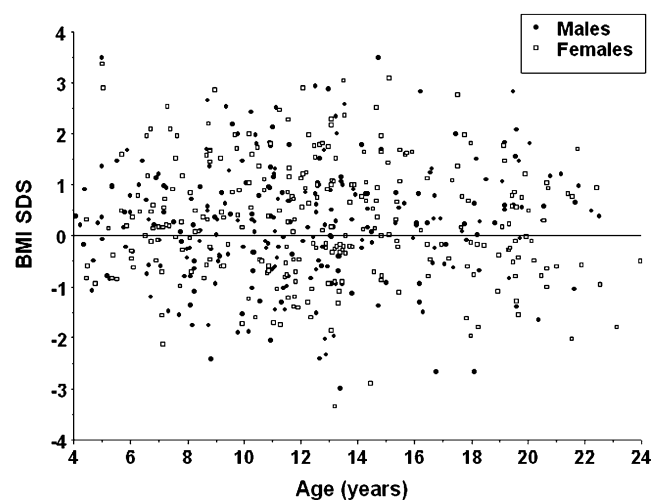


FIGURE 1. BMI SD score (BMI SDS) in the whole sample, by sex.

densitometry in association with the Lohman values for the pediatric version of Siri's equation (3, 6, 22). We also calculated the proportion of our sample that would be incorrectly categorized as obese on the basis of % fat [using cutoffs of 20% fat for males and 30% for females (23)] if using the Lohman density values and our own empirical values as the reference. All analyses were conducted with the use of Data Desk version 6.1 (Data Description Inc, Ithaca, NY).

Finally, we evaluated the agreement between hydrometry and densitometry used as 2-component techniques (ie, by using data on TBW or density in combination with our new reference values for hydration or density, respectively) and DXA. Age- and sex-specific values for lean tissue hydration and density were used to convert TBW to lean mass and density to % fat. Bland-Altman analysis was then used to compare agreement in lean mass and % fat between DXA and hydrometry or DXA and densitometry. The DXA lean mass value as used here included both bone and lean soft tissue. Correlations were also calculated to assess the association between bias and the magnitude of lean mass or % fat.

RESULTS

Valid data were obtained for 533 individuals. Data for 32 individuals were discarded in cases in which one or more of the measurements was unsuccessful ($n = 16$; mostly very young

children) or if the modeling was unsuccessful ($n = 16$) indicated by spurious body-composition data. As indicated in **Figure 1**, a wide range of BMI SDS was apparent at all ages. There was no significant correlation between BMI SDS and age in either sex.

Data on the anthropometric SDS values and the range of % fat by sex are provided in **Table 1**. On average, our sample was heavier, taller, and relatively heavier for their height than the UK reference data of the early 1990s ($P < 0.005$ in all cases). Females had significantly greater % fat than males by the 4C model ($P < 0.0001$, adjusted for age). The prevalence of obesity was 11.5% and 14.7% in males and females, respectively, and was uncorrelated with age. The numbers by pubertal stage were as follows: males: 98, 60, 28, 24, 50 (for pubertal stages 1–5, respectively); females: 87, 48, 34, 22, 80 (for pubertal stages 1–5, respectively); and 2 others not recorded. Age- and sex-specific empirical mean (\pm SD) values for lean tissue properties, stratified in 2-y age groups, are given in **Table 2**.

Our values for density and hydration with equivalent data from other studies for each sex are compared in **Figure 2**. Density increased with age in both sexes, but in different patterns. Whereas the increase was relatively consistent in females, in males the values plateaued between 10 and 15 y, and again from 18 y. Hydration decreased with age in both sexes, but again differing by sex. The decrease in hydration in males lagged behind that for females, until a sudden marked drop from ≈ 16 y. Overall, age changes in lean tissue properties were more linear in females compared with males, but in both sexes the trend was nonlinear. In comparison with previously reported data, our hydration data fit fairly well for males but not for females, in whom the simulations produced values $\approx 2\%$ too high in mid-childhood. For density, our values are systematically higher than previously modeled values throughout childhood and adolescence. Our empirical % fat values compared with those obtained by using values for body density in combination with the Lohman values for lean tissue density are shown in **Figure 3**. The mean bias was -2.1% (limits of agreement $\pm 4.7\%$), which was significantly greater in females than males (-3.3 compared with -1.1% , $P < 0.001$). The bias increased with age in males ($r = 0.30$, $P < 0.0001$) but not females ($r = 0.11$). By using cutoffs of 20% fat in males and 30% fat in females, this error would result in 24 males and 17 females being incorrectly categorized as nonobese, lowering the prevalence of obesity from 36.6% to 27.5% in males and from 33.4% to 27.2% in females.

TABLE 1

Summary statistics for anthropometric measurements and weight status by sex¹

	Males ($n = 261$)		Females ($n = 272$)	
	Mean \pm SD	Range	Mean \pm SD	Range
Weight SDS	0.31 ± 1.08	−2.42, 3.44	0.42 ± 1.10	−2.75, 3.46
Height SDS	0.21 ± 0.96	−2.09, 3.28	0.34 ± 1.02	−2.77, 3.42
BMI SDS	0.24 ± 1.15	−2.99, 3.49	0.31 ± 1.15	−3.33, 3.32
% Fat (4-component model) ²	19.2 ± 8.0	4.9, 45.5	27.0 ± 8.0	11.7, 46.9
Overweight (%)	9.2		10.7	
Obese (%)	11.5		14.8	

¹ SDS, SD score.

² Significantly different between sexes, $P < 0.0001$ (multiple regression analysis to assess significance of female sex, adjusted for age).

TABLE 2

Age- and sex-specific values for the density and hydration of lean tissue

Age	Males (<i>n</i> = 261)			Females (<i>n</i> = 272)		
	<i>n</i>	Density	Hydration	<i>n</i>	Density	Hydration
		kg/L	%		kg/L	%
4–5.99 y	22	1.0826 ± 0.0080	76.6 ± 2.4	13	1.0821 ± 0.0070	77.3 ± 2.0
6–7.99 y	24	1.0865 ± 0.0064	76.1 ± 1.8	35	1.0899 ± 0.0058	75.3 ± 1.7
8–9.99 y	40	1.0887 ± 0.0049	75.8 ± 1.4	31	1.0905 ± 0.0058	75.2 ± 1.7
10–11.99 y	51	1.0917 ± 0.0043	75.2 ± 1.4	43	1.0926 ± 0.0052	75.0 ± 1.7
12–13.99 y	51	1.0914 ± 0.0055	75.2 ± 1.6	54	1.0951 ± 0.0051	74.8 ± 1.5
14–15.99 y	18	1.0923 ± 0.0066	75.0 ± 1.6	32	1.0996 ± 0.0050	74.1 ± 1.5
16–17.99 y	23	1.0992 ± 0.0055	73.1 ± 1.6	19	1.1021 ± 0.0041	73.7 ± 1.7
18–19.99 y	21	1.0995 ± 0.0065	73.4 ± 2.0	26	1.1034 ± 0.0056	73.5 ± 1.5
20–22.99 y	11	1.1013 ± 0.0055	73.6 ± 1.5	19	1.1037 ± 0.0057	73.7 ± 1.0

The association between residual hydration or density (the actual value minus the average value for that age-sex group) and BMI SDS was weak but significant (hydration: $r = 0.26$, $P < 0.0001$; density: $r = -0.14$, $P = 0.002$). With skinfold thickness, with adjustment for age, the associations were weaker (hydration: $r = 0.11$, $P = 0.007$; density: $r = -0.05$, $P = 0.2$). Fitting a squared BMI term increased the r^2 values only slightly, indicating that the effect of BMI SDS on lean tissue properties is broadly consistent across the range of BMIs. Dividing the sample into those above and below a BMI SDS of zero, dif-

ferences in density and hydration were greater at older ages, especially in males (**Figure 4**). Mean (\pm SE) values for hydration were $1.0 \pm 0.2\%$ ($P < 0.0001$) greater, and density was 0.0022 ± 0.0007 kg/L ($P < 0.002$) lower in obese compared with nonobese individuals.

Regression equations for density and hydration on age, sex, and BMI SDS are given in **Table 3**. Age squared did not contribute significantly, which indicated a linear association. The sex-combined equation explained around half of the variance in density but only a third of the variance in hydration. Given the

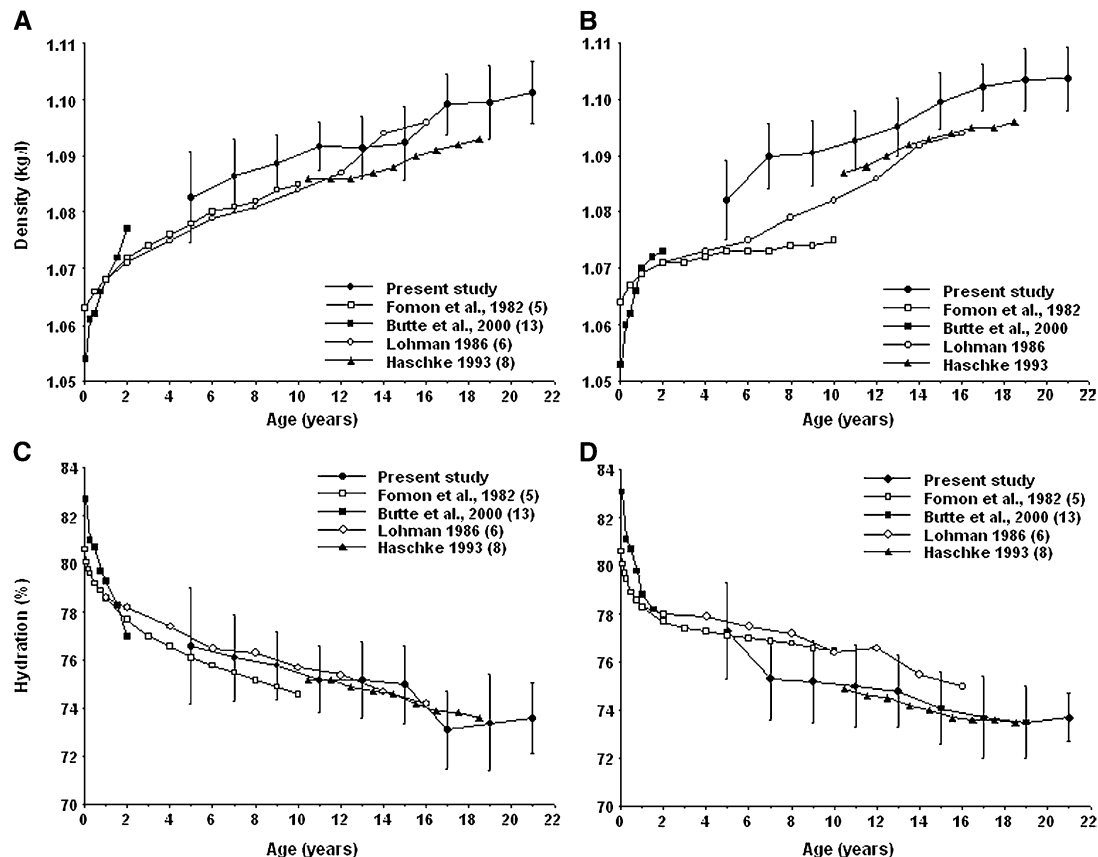


FIGURE 2. Comparison of our empirical values for lean tissue properties with those reported from the literature previously (5, 6, 8, 13) by using the mean and SD of 2-y age groups. Density of lean tissue in males (A) and in females (B). Hydration of lean tissue in males (C) and in females (D).

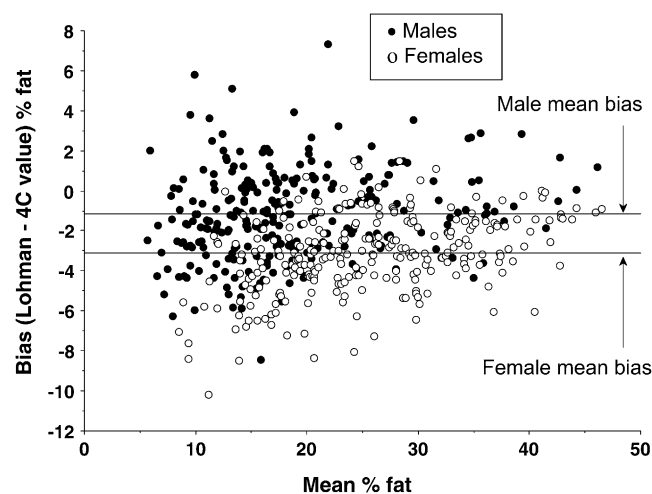


FIGURE 3. Bland-Altman plot showing the bias in % fat obtained by using body density from air-displacement plethysmography in combination with the lean tissue density values of Lohman (6) in comparison with our empirical 4-component (4C) values as the reference. The mean bias is shown for each sex.

sex differences in the age pattern of density and hydration, sex-specific models were also fitted, with similar r^2 values as those for the sex-combined model. Ethnicity (black or Asian) was not significant for hydration or density, except for black females in whom the mean density of lean tissue was 0.0003 kg/L lower than in white females ($P < 0.0008$). Excluding the effects of age

and sex, the correlations of BMI SDS with hydration and density were 0.26 ($P < 0.0001$) and -0.15 ($P < 0.001$), respectively.

Age- and sex-specific constants on the basis of our data, obtained from the M curve of the LMS method, are shown in **Table 4**. These values are for hydration of lean tissue and the C1 and C2 constants for the densitometric equation predicting % fat from body density. The values for adult males are similar to Siri's values of 4.95 for C1 and 4.50 for C2. However, for adult females our values are slightly lower: 4.90 for C1 and 4.44 for C2 at 20 y. The difference between our adult female equation and that of Siri's is 1.1% fat.

Agreement between DXA and hydrometry or densitometry as 2-component techniques is shown in **Table 5**. There was a significant underestimation of lean mass by DXA relative to both hydrometry and densitometry in females ($P < 0.001$) but not in males. Likewise, there was a significant overestimation of % fat by DXA relative to hydrometry and densitometry in females ($P < 0.001$) but not in males. The limits of agreement were approximately ± 3.1 – 3.4 kg of lean mass and ± 6.1 – 6.6% fat. Bias was in most cases associated with the magnitude of lean tissue or % fat.

DISCUSSION

This study provides the first comprehensive empirical data on the density and hydration of lean tissue, and their variability, across the majority of the growth period. These data will improve the accuracy of hydrometry and densitometry when applied to the

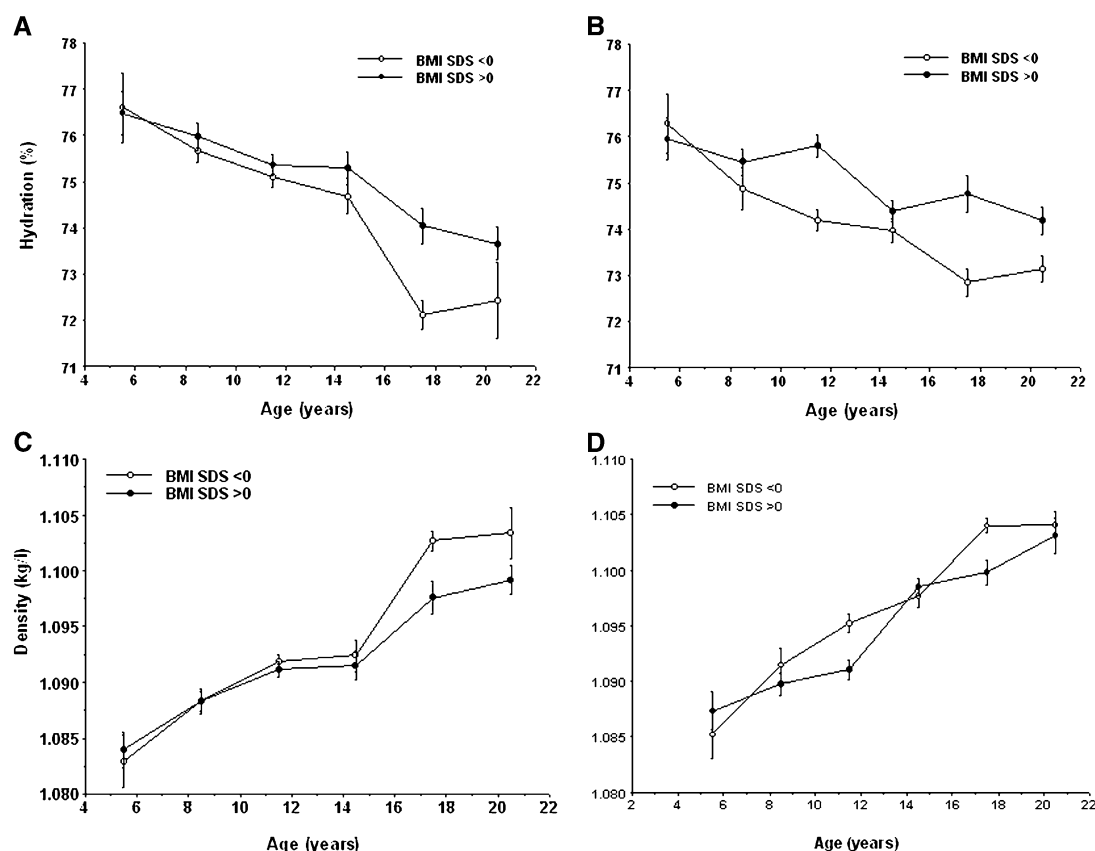


FIGURE 4. Mean (\pm SD) age changes in the properties of lean tissue, stratified according to BMI SD score (BMI SDS) above or below zero. Hydration of lean tissue in males (A) and in females (B). Density of lean tissue in males (C) and in females (D).

TABLE 3Regression equations for the prediction of lean tissue density and hydration by age, sex, and BMI SD score (BMI SDS)¹

	Density (×1000)					Hydration				
	B	SE	<i>t</i>	<i>P</i>	<i>r</i> ²	B	SE	<i>t</i>	<i>P</i>	<i>r</i> ²
All										
Constant	1074.8	0.97	1106	<0.0001	0.52	77.59	0.28	281	<0.0001	0.30
Age (y)	1.18	0.05	22.0	<0.0001		−0.19	0.01	−12.7	<0.0001	
Female sex	2.79	0.48	5.8	<0.0001		−0.27	0.14	−2.0	0.046	
BMI SDS	−0.90	0.21	−4.3	<0.0001		0.41	0.06	7.0	<0.0001	
Males										
Constant	1078.2	1.04	1037	<0.0001	0.44	77.71	0.30	261	<0.0001	0.31
Age (y)	1.14	0.08	14.0	<0.0001		−0.23	0.02	−9.7	<0.0001	
BMI SDS	−1.09	0.30	−3.6	0.0004		0.43	0.09	4.9	<0.0001	
Females										
Constant	1079.8	0.98	1093	<0.0001	0.53	76.69	0.28	277	<0.0001	0.28
Age (y)	1.23	0.07	17.1	<0.0001		−0.17	0.02	−8.2	<0.0001	
BMI SDS	−0.70	0.28	−2.4	0.015		0.42	0.08	5.3	<0.0001	

¹ In each model, hydration or density was regressed on predictors that included age, BMI SDS, and female sex as specified.

pediatric population. They also reveal important nonlinear patterns of chemical maturation in children, which further differ between the 2 sexes. Our empirical values were similar to those of Lohman (6) for hydration in males but differed substantially for hydration in females and for density in both sexes. These differences generate mean errors of approximately −1 to −2.5% fat in individuals of average fatness according to age, with an overall mean bias of −2.1% for densitometry. Such errors are clinically significant when, for example, categorizing obesity according to adiposity [% fat: >20% in males, 30% in females (23)] rather than BMI.

Maturation changes in hydration and density have been attributed to a combination of factors. On the one hand, decreases

in the water content of lean tissue correspond to increases in protein and mineral content (5) and hence to the ratio of extracellular solids to body water (24). However, this absolute decrease in water content also incorporates a decline in the ratio of extracellular to intracellular water, as cells increase in size and thus in their relative water content (24). It has been generally assumed that these changes occur fastest in early childhood, and that the rate of change is slower after 2 y (5, 13). Our data, however, suggest that chemical maturation continues at a faster rate until ≈7 y of age and that less maturation takes place during adolescence than previously assumed.

Our values for male adults aged 18–23 y are extremely similar to those generally assumed, with a mean hydration of 73.4–73.6%

TABLE 4Median values for hydration, density, and constants (C1 and C2) for the pediatric version of Siri's (3) equation, obtained by using the LMS (lambda, mu, sigma) method¹

Age	Males				Females			
	Hydration	Density	C1	C2	Hydration	Density	C1	C2
	%	kg/L			%	kg/L		
5 y	76.5	1.0827	5.36	4.95	76.7	1.0837	5.33	4.92
6 y	76.3	1.0844	5.32	4.90	76.1	1.0865	5.27	4.85
7 y	76.1	1.0861	5.28	4.86	75.5	1.0887	5.22	4.79
8 y	75.9	1.0877	5.24	4.82	75.2	1.0900	5.19	4.76
9 y	75.7	1.0889	5.21	4.79	75.1	1.0909	5.17	4.74
10 y	75.5	1.0900	5.19	4.76	75.0	1.0916	5.15	4.72
11 y	75.3	1.0911	5.16	4.73	75.0	1.0924	5.13	4.70
12 y	75.2	1.0917	5.15	4.72	74.9	1.0937	5.10	4.67
13 y	75.0	1.0920	5.14	4.71	74.6	1.0954	5.07	4.63
14 y	74.8	1.0927	5.13	4.69	74.4	1.0975	5.02	4.58
15 y	74.4	1.0942	5.09	4.66	74.1	1.0996	4.98	4.53
16 y	74.0	1.0960	5.05	4.61	73.8	1.1011	4.95	4.49
17 y	73.7	1.0978	5.02	4.57	73.7	1.1020	4.93	4.47
18 y	73.5	1.0991	4.99	4.54	73.6	1.1027	4.92	4.46
19 y	73.4	1.1000	4.97	4.52	73.6	1.1031	4.91	4.45
20 y	73.3	1.1006	4.96	4.51	73.6	1.1035	4.90	4.44

¹ C1 is calculated as $(D_L \times D_F)/(D_L - D_F)$, and C2 is calculated as $D_F/(D_L - D_F)$; D_L and D_F represent density of lean tissue and density of fat, respectively. % Fat is calculated as $[(C1/BD) - C2] \times 100$, where BD is measured whole-body density.

TABLE 5

Bland-Altman analysis of the agreement between dual-energy X-ray absorptiometry and hydrometry or densitometry as 2-component techniques

	Sex	Bias ¹	Limits of agreement ²	P ³	r ⁴	P ⁵
Hydrometry						
Lean mass	Male	-0.16	±3.44	0.1	-0.25	<0.001
	Female	-1.05	±3.28	<0.001	-0.51	<0.001
% Fat	Male	0.1	±6.6	0.6	0.31	<0.001
	Female	1.3	±3.3	<0.001	0.41	<0.001
Densitometry						
Lean mass	Male	-0.10	±2.68	0.2	-0.33	<0.001
	Female	-1.08	±3.11	<0.001	-0.51	<0.001
% Fat	Male	-0.1	±6.3	0.7	-0.02	0.9
	Female	1.6	±6.1	<0.001	0.08	0.1

¹ Calculated as dual-energy X-ray absorptiometry value minus hydrometry or densitometry value.² Calculated as twice the SD of the bias.³ Refers to significance of bias.⁴ Correlation between bias and magnitude of lean mass or % fat.⁵ Refers to significance of *r*.

and a density of 1.0995–1.1013 kg/L. Our female values are similar for hydration (73.5–73.7%) but slightly greater for density (1.1034–1.1037 kg/L). The average value from 9 cadaver studies was 73.7%, although the range was quite high (68.6–80.8%) (25), whereas the study by Fuller et al (19), using the 4C model, reported mean (\pm SD) values of $73.8 \pm 2.1\%$ (19). Our adult values for density are likewise very close to the value of 1.10 kg/L assumed by Siri (3) and Brozek et al (26) and to the mean (\pm SD) provided by 8 cadaver studies of 1.099 ± 0.015 kg/L (27). From their modeling, Wang et al (27) predicted ≈ 0.002 – 0.004 kg/L greater density in women compared with men, and our empirical data show very similar differences of 0.004 kg/L at age 18–19.99 y and 0.002 kg/L at age 20–22.99 y. Consequently, our data imply that there should be slightly different versions of Siri's equation for adult men and women to calculate body composition from whole-body density.

Although in the past decade many body-composition researchers have selected DXA for pediatric research studies, significant bias has been shown in this technique, which further varies between instruments, and in relation to sex and nutritional status (28–30). Limits of agreement are also wide in individuals. In the present study, hydration and density have been calibrated against the 4C model, thus removing any average biases at each age. By using this calibration, we found that DXA showed significant disagreement with hydrometry and densitometry as 2-component techniques in females but not males, which is similar to our previous evaluation of DXA by using the 4C model (29).

However, our study further shows that nutritional status, as indexed by BMI SDS or skinfold thickness, accounts for some of the between-individual variability in lean tissue properties after adjustment for age and sex. This finding is consistent with previous studies by us (14) and others (12) and is partly explained from a theoretical perspective by the fact that hydration of the cellular component of adipose tissue is greater than that of lean tissue (25). In males, this effect of BMI is negligible until late adolescence, but in females it is significant during pubertal development from ≈ 10 y old, although it temporarily lessens at ≈ 14 y. Failure to take this effect into account will therefore introduce bias in the final body-composition values in relation to

nutritional status, whether hydrometry or densitometry is used. A correction factor could be applied on the basis of our linear regression of hydration or density against BMI SDS, and in the absence of such correction errors of 2–4% fat could arise.

The increase in hydration in obese compared with nonobese individuals averaged $\approx 1\%$ after adjustment for age and sex, but in other studies reached $\approx 2\%$ in extreme obesity (14). This greater increase in hydration has been attributed to expansion of the extracellular water pool (31). The density of lean tissue is likewise reduced by ≈ 0.015 kg/L in obese individuals, as we have found previously (32). When high levels of accuracy are required, 2-component techniques are inherently limited because of their assumption of constant values for lean tissue composition, and multicomponent models are preferred in obese individuals, in whom variability in lean tissue properties is also greater.

Nevertheless, we have previously shown that around half of the variability in hydration and density of lean tissue in the normal weight range can be attributed to methodologic imprecision (10). This is likely to account for the limited success (r^2 values ranging from 0.28 to 0.53) with which factors such as age, sex, and BMI SDS accounted for density and hydration variance in our regression models. From a theoretical perspective, a narrow range of hydration variability is predicted in healthy individuals of a given age because of limited capacity for variability in cellular and extracellular hydration and in the ratio of extracellular solids to TBW (25). Because mineral and protein content are closely related, the scenario for hydration is broadly applicable to density.

Disease can generate marked shifts in the extra- to intracellular ratio and change hydration and density to a greater extent. In pediatric survivors of acute lymphoblastic leukemia, we detected a 1.8% increase in hydration and a 0.007 -kg/L reduced density relative to healthy control children (33), some of which may be attributed to the greater adiposity of the patients. In healthy individuals, however, these tissue properties have a limited range of biological variability, and among the range of body-composition techniques, hydrometry and densitometry remain relatively accurate and perform well when compared against the gold-standard

4C model (10, 34, 35). Furthermore, if the SD of hydration and density in patients is similar to that in healthy children, as we found in our study of leukemia survivors (33), a simple correction factor might be used in subsequent evaluation of such patients on the basis of 2-component methodologies.

Due to practical factors, we were not able to extend our study to children aged <4 y. Body density is rarely measured in young children, but there are negligible difficulties in applying hydrometry across the entire human age range. Butte et al (13) recently published reference values for hydration in the first 2 y of life, although their multicomponent model calculations did not match exactly with our own due to the difficulty of measuring body density in very young age groups. The link between the 2 data sets is not very smooth in females, but it remains unclear if this is an effect of small sample size or methodologic issues.

A further limitation is that we had limited capacity to investigate possible ethnic variability in lean tissue properties. Over 90% of our sample was white, and our nonwhite individuals were distributed across a range of ethnic categorizations. Others have previously reported differences in lean tissue hydration between ethnic groups in adults (35), although the evidence for density is inconsistent (35–38), but findings in children have generally been negative (7, 12). Modeling suggests that some ethnic differences in density may be too small for empirical detection (27), and our sample was able to detect only a small difference in the density of lean tissue in black females. This issue therefore requires further investigation; however, the differences identified in adult studies were relatively small, and neither hydrometry nor densitometry are likely to be seriously compromised in accuracy by failing to take ethnicity into account, unless relatively small differences between ethnic groups are themselves the focus of study.

In conclusion, our study provides the first empirical reference data on lean tissue properties for childhood and adolescence and should improve the accuracy of hydrometry and densitometry in relatively healthy members of the pediatric population. Nutritional status accounts for variability in these properties over the normal range of BMI, but the error resulting from this source is of moderate magnitude until individuals are relatively obese. Two-component techniques may be less appropriate in individuals with obesity or more severe disease states, in whom multi-component models remain the preferred approach for molecular data on body composition.

The authors' responsibilities were as follows—JCKW and MSF: study design; JEW, SC, DH, KK, CG, and CW: data collection and modeling; TD: mass spectrometric analysis; JCKW and TJC: statistical analysis with TJC; and JCKW: writing of first draft of the manuscript. All authors contributed to subsequent discussions and revisions. None of the authors declared a conflict of interest.

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Body composition assessed by the 4-component model and association with lung function in 6–12-y-old children with cystic fibrosis^{1–3}

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ABSTRACT

Background: Malnutrition is an indicator of a poor prognosis in patients with cystic fibrosis (CF). Previous body-composition (BC) studies in children with CF used 2-component models (2CMs) to assess fat mass (FM) and fat-free mass (FFM), but to our knowledge no study has used the gold-standard 4-component model (4CM), which allows for a more accurate evaluation of the nature of both elements.

Objective: We measured BC by using the 4CM in 6–12-y-old children with CF to 1) compare findings with those of healthy, matched control children and reference data; 2) relate BC to lung spirometry [forced expired volume in 1 s (FEV₁)]; and 3) compare findings with those from more commonly used 2CM techniques.

Design: One hundred clinically stable children with CF (57% girls) aged 6–12 y were measured by using the 4CM. Children with CF underwent spirometry (FEV₁).

Results: Girls with CF had significantly less FM than did healthy girls, even after adjustment for height and pubertal status; boys with CF had higher body mass index SD scores than did healthy boys. FM in girls was positively associated with the FEV₁ percentage predicted. The 2CM FM was significantly different from the 4CM FM, with differences dependent on sex and condition, although most techniques identified a relation between FM and FEV₁ in girls.

Conclusions: Although shorter than healthy children, boys with CF were heavier and had a BC within the normal range; however, girls with CF had lower FM than did healthy girls, and this was associated with poorer lung function. Given the worse prognosis in girls, this finding merits more attention. The reliability of 2CM techniques varied with sex and health status. *Am J Clin Nutr* 2010;92:1332–43.

INTRODUCTION

Cystic fibrosis (CF) is a lethal, autosomal, recessive genetic disorder that is most common in white individuals. Historically, it has been considered that children with CF do not grow normally because of a negative energy balance caused by the detrimental effects of the difficulty in breathing, chronic infection and inflammation, decreased absorption, and increased nutritional loss (1). When growth has been suboptimal, there is a negative effect on physical activity, appetite, and lung function (2). However, recent research suggests that growth in children with CF has improved (3, 4). It is also apparent that with improvements in treatment, life expectancy has increased considerably, and

complications such as osteoporosis have become apparent in young adults. Consequently, there is an increased focus on longer-term health as well as short-term health and survival.

Typically, the growth of children with CF is monitored by using height, weight, and body mass index (BMI) measurements, but these techniques do not give any indication of the nature of body composition. The investigation of body composition is beneficial because it aids understanding of the disease process, allows for assessment of the effectiveness of medical and nutritional interventions, and may identify those children most at risk of deterioration. Many previous studies used techniques with inherent problems related to poor precision or assumptions about the nature of the fat-free mass (FFM) (5) or inadequate sample size. In addition, techniques suitable for healthy children are predicted to be biased when applied to patients (6, 7). The gold-standard 4-component model (4CM) of body composition, which was used in this study, allows for a more accurate quantification of fat mass (FM) and assessment of the nature of FFM (water, protein, and mineral) because actual measurements of components of body composition are made. In patients with CF, it is important to establish whether specific components of body composition are independent predictors of clinical outcomes because this would have implications for treatment regimens.

To our knowledge, no previous study of body composition in children with CF has adopted the 4CM. The aims of this study were to 1) compare young children with CF to their healthy counterparts by using the 4CM, 2) examine associations between body composition and lung function in children with CF, and 3) compare the

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findings from the gold-standard 4CM to those from commonly used 2-component model (2CM) techniques.

obtain the actual BV. All measurements were made by one of 2 operators (JEW and CMW).

SUBJECTS AND METHODS

Subjects

Recruitment started in October 2002 for children with CF aged 6–12 y under the care of Great Ormond Street Hospital for Children (London, United Kingdom) who were clinically stable and emotionally able to undergo measurements. The children with CF were diagnosed on the basis of confirmatory genetics or a positive sweat test with sodium and chloride concentrations > 60 mmol/L. Healthy children for matched-pair and cross-sectional reference comparison were recruited for another study of body composition at the Institute of Child Health (London, United Kingdom) that started in February 2002. Measurements were conducted over a 90-min visit to the study center at Great Ormond Street Hospital for Children after consumption of a light meal. Ethical permission was obtained from the ethical committee of the Institute of Child Health. Written consent was obtained from parents and written assent from children aged ≥ 11 y; verbal assent was obtained from children aged < 11 y.

Dual-energy X-ray absorptiometry

Bone mineral content (BMC), bone mineral density (BMD), and bone area (BA) were measured with a Lunar Prodigy whole-body scanner (GE Medical Systems, Slough, United Kingdom) in conjunction with software v.12.1 (2008, Lunar Prodigy; GE Medical Systems). All scans were performed with the subject wearing light indoor clothing and with no removable metal objects present. The typical scan time was 5 min depending on height. The radiation exposure per whole-body scan was estimated to be $2 \mu\text{Sv}$, lower than the daily background level. All scans were performed by one operator (CMW). The precision of bone density, which was established by repeated measurements of phantoms on 6 successive days, was $< 2\%$. The precision of soft tissue analysis for a Lunar DPX-L instrument (regarded by the manufacturer, GE Medical Systems, as similar to our own model) established by repeated measurements of humans on 4 successive days was reported as 1% for FFM and 2% for FM (8).

Body volume

Body volume (BV) was measured by using air-displacement plethysmography (ADP) (Bodpod Instrumentation; Life Measurement Instruments, Concord, CA) according to the manufacturer's instructions as previously described (9). Measurements were made while the subject wore a close-fitting swimming costume and hat. The raw-volume values that appeared transiently on the screen were recorded, and to improve precision, the procedure was repeated until 2 mean values for raw density of within 0.007 kg/L were obtained (10). Where it was not possible to achieve 2 such measurements because of breathing irregularities in 3 of the CF children, the mean of all raw volume values was used after values ± 2 SDs were discarded (achieved over 4 separate tests of a minimum of 8 and maximum of 12 volumes). An adjustment of the mean raw volume was then made by using the predicted lung volume (11, 12) and surface area (13) to

Anthropometric measures

Body weight was measured as an integral stage of the Bodpod Instrumentation procedure (Life Measurement Instruments) to $\leq 0.01 \text{ kg}$ with the child dressed in a swimsuit. Accuracy was confirmed by the use of 2 solid weights of known mass. Standing height was measured to $\leq 0.1 \text{ cm}$ with a wall-mounted digital display stadiometer (Holtain, Dyfed, United Kingdom). BMI was calculated as weight in kilograms divided by the square of height in meters. Weight, height, and BMI were converted to SD scores (SDSs) by using UK 1990 reference data (14, 15). Waist, hip, and midupper arm circumference were measured with a fiberglass tape, and bicep, tricep, and subscapular and suprailiac skinfold-thickness measurements were taken in triplicate to the nearest 0.2 mm and averaged. Measurements were taken on the left sides of subjects according to the method of Lohman et al (16) with a Holtain skinfold-thickness caliper.

Deuterium dilution

Total body water (TBW) was assessed by ^2H -labeled water dilution by using a dose equivalent to $0.05 \text{ g } ^2\text{H}_2\text{O/kg}$ body weight (99.9 atom percentage excess). Doses were diluted with water to $\approx 100 \text{ mL}$. Saliva samples were taken predose and 4 h postdose with absorbent cotton swabs (Sarstedt, Rommelsdorf, Germany) ≥ 30 min after the last ingestion of food or drink. Deuterium samples were analyzed by Iso-Analytic Ltd (Sandbach, United Kingdom) by using the equilibration method of Scrimgeour et al (17). Briefly, 0.3 mL liquid, along with a vial of 5% platinum on alumina powder (Sigma-Aldrich, Poole, United Kingdom), was placed in a septum sealed container (Labco, High Wycombe, United Kingdom) and flushed for 2 min with hydrogen. Low-enrichment and high-enrichment standard waters were similarly prepared to normalize data against Standard Mean Ocean Water-Standard Light Antarctic Precipitation standards. Samples equilibrated at room temperature for 3 d before analysis. The head spaces in the containers were analyzed for deuterium enrichment on a continuous flow-isotope ratio mass spectrometer (Geo20-20; Europa Scientific, Crewe, United Kingdom). The accuracy of analyses was checked by measuring an intermediate water standard within each batch of samples. All samples were prepared and analyzed in duplicate. The mean SD of deuterium analyses by the equilibration technique in the laboratory was $< 2.5\%$. The ^2H -dilution space was assumed to overestimate TBW by a factor of 1.044 (18), and correction was made for fluid intake during the equilibrium period to derive actual body water.

2CMs

2CMs of body composition, which distinguished FM and FFM, were calculated as follows.

Anthropometric measures

Percentage body fat was estimated from skinfold-thickness measurements by using the age- and sex-specific equations of Slaughter et al (19) (tricep and subscapular) and Deurenberg et al

(20) (tricep, bicep, subscapular, and suprailiac). FM was calculated as

$$(\text{Percentage fat} \times \text{weight})/100 \quad (1)$$

Hydrometry

TBW was used to calculate FFM, with assumption for an age- and sex-specific hydration factor of FFM, (HF^{FFM}) according to published values of Lohman (21).

$$FFM = TBW/HF^{FFM} \quad (2)$$

FM was calculated as

$$FM = \text{weight} - FFM \quad (3)$$

and

$$\text{Percentage fat} = FM/\text{weight} \times 100 \quad (4)$$

Dual-energy X-ray absorptiometry

FM and lean-mass (LM) values were obtained from a whole-body dual-energy X-ray absorptiometry (DXA) scan as described, and FFM was calculated as

$$LM + BMC \quad (5)$$

Densitometry

Whole-body density was calculated from BV (which was adjusted for residual lung volume and surface-area artifact) and weight by using ADP (Bodpod Instrumentation; Life Measurement Instruments).

$$\text{Body density} = \text{weight}/BV \quad (6)$$

$$\text{Percentage fat} = (527/\text{body density}) - 485 \quad (22) \quad (7)$$

and

$$FM = (\text{percentage fat} \times \text{weight})/100 \quad (8)$$

4CM

The 4CM uses values of BMC, body weight, BV, and TBW to derive values for mineral, water, fat, and protein as described previously (22, 23). Assumed densities of the 4 components were accounted for when calculating FM from the measurements

$$FM \text{ (in kg)} = [(2.747 \times BV) - (0.710 \times TBW)] + [(1.460 \times BMC) - (2.050 \times \text{body weight})] \quad (9)$$

and FFM was calculated as the difference between weight and FM.

Pubertal status

Pubertal stage was self-assessed with line drawings that showed the different Tanner stages for breast or genital development (24). For the purposes of analyses, prepubertal (stage 1) was considered distinct from pubertal (stages 2–5).

Physical activity

Physical activity levels were assessed by asking the parent to give a rating of the child's activity as follows: 1) much less than peers, 2) less than peers, 3) same as peers, 4) more than peers, and 5) much more than peers. Because of small numbers, the lower 2 and upper 2 categories were combined and resulted in the following 3 groups: less than, same as, and more than peers.

Lung function

Laboratory spirometry [forced expired volume in 1 s (FEV_1)] was measured according to laboratory protocols on the basis of American Thoracic Society and European Respiratory Society standards for spirometry (25) adapted for children (26). Values for analyses were calculated as the FEV_1 percentage predicted compared with a large reference population (27). Children with $FEV_1 < 45\%$ were classified as having severe impairment, children with $FEV_1 \geq 45$ and $< 65\%$ were classified as having moderate impairment, children with $FEV_1 \geq 65\%$ and $< 85\%$ were classified as having mild impairment, and children with $FEV_1 \geq 85\%$ were classified as having normal lung function.

Statistical analyses

Size adjustment and SDS calculation

Characteristics of all children were compared with 1990 UK reference data to generate SDSs for weight, height, BMI, and waist circumference. A method analogous to that of BMI (weight divided by the square of height) was applied to absolute values of FM, FFM, protein mass (PM) and mineral mass (MM) to remove the effect of size; the FM index (FMI; FM divided by the square of height), FFM index (FFMI; FFM divided by the square of height), PM index (PMI; PM divided by the square of height), and MM index (MMI; MM divided by the square of height). These variables were compared with measurements performed in a contemporary reference population of 533 healthy subjects aged 4–23 y (JCK Wells, JE Williams, and MS Fewtrell, unpublished data, 2002–2007) to produce SDSs. Total and lumbar spine (LS) BMD SDSs were generated from the Lunar Prodigy software (2008, v.12.1; GE Medical Systems) by using machine reference data matched for age, sex, and ethnic group. BMD is a 2-dimensional measurement, which does not take into account bone size. Small children may have low BMC or BMD because they have small bones or less mineral than expected for the size of bone (28). To adjust for size, the bone mineral apparent density (BMAD), which is a 3-dimensional “volumetric” measurement, was calculated for the LS from the BMC and BA as follows (29):

$$BMAD = BMC/BA^{1.5} \quad (10)$$

and size-adjusted SDSs were calculated by using reference data collected for the GE Lunar Prodigy (30).



Comparison of body-composition variables

With the use of a one-sample *t* test, comparisons were made between 1) all children and the 1990 UK reference population for anthropometric SDSs, 2) all children and the contemporary reference population for body-composition variables SDSs and by using paired sample *t* tests (3), and pair-matched children with CF and healthy children for all anthropometric measures and body-composition variables. Initial analyses indicated a strong significant difference between the sexes, and therefore, they were analyzed separately. General linear models were used to examine differences between children with CF and healthy control children by taking into account factors that predicted body-composition variables with matched pair, group and puberty as fixed factors and age and height (for nonindexed variables) as continuous variables. Because total MM is predominantly bone mineral, MM was also adjusted for bone area to account for the effect of bone size.

Lung spirometry

Simple regression analyses were used to investigate the relation between body-composition variables and lung spirometry (FEV₁ percentage predicted) in children with CF. Height was included in the model.

Comparison of 2CMs and 4CM

The method of Bland and Altman (31) was used to assess the agreement between the values for FM by 4 2CMs and the criterion 4CM. The mean difference between techniques (bias; 2CM – 4CM) and the ± 2 SDs of the difference between

techniques (limits of agreement) were calculated. The bias was tested for significance from zero by using a paired *t* test. To express the bias as a percentage of the mean, the bias of the individual natural log value $\times 100$ was calculated (32). The extent to which the magnitude of the bias was related to the magnitude of the variable was calculated as the correlation between the difference and the mean of the measured values. All analyses were performed with the Statistical Package for Social Sciences 18.0 software (SPSS Inc, Chicago, IL), and $P < 0.05$ was considered significant.

RESULTS**Subjects**

One hundred of 116 eligible children with CF were recruited, and complete 4CM measurements were obtained in 90 children [4 children (3 girls) refused to enter the Bodpod air-displacement plethysmography instrumentation (Life Measurement Instruments), and 6 postdose isotope dilution samples had inadequate volumes for analysis (4 girls)]. However, a body-water calculation was implausible for 5 children (3 girls); therefore, pair matching with healthy age-, sex-, and ethnicity-matched control children was made for 85 CF children [boys: 37 (44%); girls: 48 (56%)]. Characteristics of the children are shown in **Table 1**. The self-assessed pubertal status for the 170 children was prepubertal for boys [boys with CF: 34 (92%); control boys: 31 (71%)] and girls [girls with CF: 34 (71%); control girls: 29 (60%)]. All subjects with CF, except one girl, were pancreatic insufficient and had a wide range of pulmonary disease with

TABLE 1

Characteristics of cystic fibrosis (CF) and control children in case-control analyses ($n = 85$)¹

	CF boys ($n = 37$)	CF girls ($n = 48$)	Control boys ($n = 37$)	Control girls ($n = 48$)
Age (y)	9.28 \pm 0.92 ²	9.55 \pm 1.62	9.28 \pm 1.08	9.57 \pm 1.78
Weight (kg)	30.2 \pm 6.64	30.0 ^c \pm 7.19	30.1 \pm 6.38	34.6 \pm 9.36
Height (m)	1.31 \pm 0.08	1.32 ^c \pm 0.11	1.35 \pm 0.09	1.37 \pm 0.11
BMI (kg/m ²)	17.3 ^b \pm 2.23	16.4 ^c \pm 1.99	16.4 \pm 1.79	18.1 \pm 2.84
Weight SDS	−0.01 \pm 1.05	−0.56 ^{cf} \pm 1.04	0.02 \pm 0.76	0.42 ^c \pm 0.98
Height SDS	−0.54 ^d \pm 1.04	−0.59 ^{cf} \pm 1.12	0.06 \pm 0.86	0.22 \pm 0.87
BMI SDS	0.43 ^{be} \pm 0.97	−0.33 ^{cd} \pm 0.95	−0.03 \pm 0.85	0.39 ^d \pm 1.10
Waist (cm)	63.5 ^a \pm 6.25	60.2 \pm 5.5	58.9 \pm 4.18	62.2 \pm 7.67
Waist SDS	1.16 ^{af} \pm −0.79	0.69 ^f \pm 0.78	0.40 ^c \pm 0.63	0.94 ^f \pm 1.05
Hip (cm)	68.4 \pm 6.37	68.2 ^c \pm 7.06	69.2 \pm 6.39	74.6 \pm 8.63
MUAC (cm)	19.9 \pm 2.53	19.7 ^c \pm 2.59	20.1 \pm 2.80	22.2 \pm 3.31
Sum of 4 skinfold thicknesses	34.7 \pm 19.8	37.1 ^b \pm 14.6	56.6 \pm 165.5	48.4 \pm 21.1
FEV ₁ percentage predicted	91.3 \pm 20.9	77.6 \pm 18.4	—	—
FVC percentage predicted	99.4 \pm 18.3	83.5 \pm 15.9	—	—
Prepubertal (%) ³	92	71	84	60
Pancreatic insufficient (n)	37	47	—	—
Liver disease (cases)	1	1	—	—
Diabetes (cases)	0	1	—	—
Ps aerug (cases)	10	11	—	—
Staph aureus (cases)	7	8	—	—

¹ SDS, SD score compared with UK reference data 1990 (14, 15); MUAC, midupper arm circumference; Sum of 4 skinfold thicknesses, bicep + tricep + subscapular + suprailiac ($n = 79$); FEV₁ percentage predicted, forced expired volume in 1 s percentage of the expected (27); FVC percentage predicted, forced vital capacity percentage of the expected (27); Pancreatic insufficient, reduced pancreatic function that required the oral addition of pancreatic enzymes; Ps aerug, chronic *Pseudomonas aeruginosa* infection of the lungs; Staph aureus, chronic *Staphylococcus aureus* infection of the lungs. ^{a,b,c}Comparisons of CF boys and control boys or CF girls and control girls (paired *t* test): ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$; ^{d,e,f}CF and control children compared with UK 1990 reference data (14, 15) (one-sample *t* test): ^d $P < 0.05$, ^e $P < 0.01$, ^f $P < 0.001$.

² Mean \pm SD (all such values).

³ Prepuberty was based on Tanner staging 1 (prepubertal) to stages 2–5 (pubertal).



a median FEV₁ of the predicted value of 85.1% with values between 32% and 131%; 8 (7 girls) subjects with CF, had a gastrostomy in situ, 2 (1 girl) subjects with CF had liver disease, and 1 girl with CF was diabetic. The genotype was homozygous $\Delta F508$ for 59 children and heterozygous $\Delta F508$ for 19 children. Five children had a non- $\Delta F508$ genotype, and 2 children were of unknown genotype. Sixteen eligible children with CF (9 girls) who were not recruited and 15 children (10 girls) who were measured and not included in the analyses were not significantly different with respect to height SDSs, weight SDSs, and BMI SDSs.

Anthropometric measures

Compared with UK reference data

Characteristics of the children are shown in Table 1. The healthy children were representative of the 1990 UK reference data apart from the girls being significantly heavier ($P < 0.01$) and both boys and girls having greater waist circumferences ($P < 0.01$). Boys with CF were significantly shorter ($P < 0.05$) but had a higher BMI SDSs ($P < 0.01$) and waist circumferences ($P < 0.01$), and girls with CF were lighter ($P < 0.001$) and shorter ($P < 0.001$) and had lower BMI SDSs ($P < 0.05$) and greater waist circumferences ($P < 0.01$) than the 1990 UK reference data.

Compared with pair-matched children

Comparisons between children with CF and healthy pair-matched control children indicated that boys with CF were not

significantly different apart from higher BMI SDSs ($P < 0.01$) and waist circumference SDSs ($P < 0.05$). The range of BMI SDSs was -1.5 to 2.4 for boys with CF and -1.9 to 2.0 for control boys. Girls with CF were significantly shorter ($P < 0.01$) and lighter ($P < 0.01$), with lower anthropometric values for all measures apart from waist circumference (non-significant), than pair-matched control girls.

Body composition

Comparisons by reference populations and case-control subjects are shown in Table 2 with a summary in Table 3.

Compared with reference data

Comparisons of CF children with a reference population (Lunar Prodigy reference data for bone and unpublished reference data for body composition) indicated higher total BMD SDSs ($P < 0.001$) and FFMI SDSs ($P < 0.01$) in boys with CF and lower LS BMD SDSs ($P < 0.001$), BMAD SDSs (size adjusted; $P < 0.01$), FMI SDSs ($P < 0.001$), FFMI SDSs ($P < 0.05$), and MMI SDSs ($P < 0.001$) in girls.

Compared with pair-matched children

Boys with CF had significantly higher total BMD SDSs ($P < 0.05$) and FFMI SDSs ($P < 0.01$) than did case-control boys. Girls with CF had less total BMD SDSs ($P < 0.05$) and LS BMD SDSs ($P < 0.001$) and significantly lower FMI SDSs ($P < 0.001$) and MMI SDSs ($P < 0.001$) than did case-control girls.

TABLE 2

Body composition of cystic fibrosis (CF) and control children used in case-matched analyses ($n = 85$ pairs)¹

	CF boys ($n = 37$)	CF girls ($n = 48$)	Control boys ($n = 37$)	Control girls ($n = 48$)
Body volume (L)	28.3 ± 6.49	$27.8^c \pm 7.09$	27.5 ± 6.51	33.6 ± 9.41
Total body water (L)	18.0 ± 3.12	$17.1^c \pm 3.72$	17.9 ± 3.71	18.9 ± 4.31
Total BMC (kg)	1.05 ± 0.21	$1.01^c \pm 0.24$	1.07 ± 0.24	1.20 ± 0.33
Total BMD (g/cm ²)	$0.90^a \pm 0.06$	$-0.87^b \pm 0.05$	0.88 ± 0.05	0.90 ± 0.07
Total BMD SDS	$0.58^{a,f} \pm 0.66$	$-0.11^a \pm 0.74$	0.23 ± 0.05	0.23 ± 0.62
LS BMD (g/cm ²)	0.73 ± 0.08	$0.73^a \pm 0.10$	0.71 ± 0.08	0.78 ± 0.11
LS BMD SDS	-0.03 ± 0.74	$-0.49^f \pm 1.12$	-0.19 ± 0.75	-0.05 ± 0.93
LS BMAD SDS	0.18 ± 0.96	$-0.40^c \pm 1.19$	$-0.12^d \pm 0.85$	-0.20 ± 1.06
4-Component model				
FM (kg)	5.95 ± 3.66	$6.41^c \pm 2.93$	5.31 ± 2.80	9.46 ± 4.71
FMI SDS	0.02 ± 1.10	$-0.76^{c,f} \pm 0.89$	-0.22 ± 0.97	0.02 ± 0.95
Fat (%)	19.0 ± 7.84	$20.6^c \pm 5.47$	17.8 ± 6.14	26.1 ± 7.46
FFM (kg)	23.5 ± 3.60	$22.7^c \pm 4.98$	23.5 ± 5.09	25.1 ± 5.60
FFMI SDS	$0.56^{b,c} \pm 0.97$	$-0.39^d \pm 1.07$	-0.14 ± 1.04	-0.08 ± 0.94
FFM hydration (%)	75.9 ± 2.07	74.9 ± 1.97	76.0 ± 1.49	75.1 ± 1.54
FFM density (kg/L)	1.087 ± 0.007	1.090 ± 0.007	1.087 ± 0.005	1.091 ± 0.005
Protein (kg)	4.31 ± 0.65	4.45 ± 1.06	4.30 ± 1.18	4.72 ± 0.99
PMI SDS	0.36 ± 1.36	0.08 ± 1.03	-0.16 ± 1.11	-0.05 ± 0.97
Mineral (kg)	1.34 ± 0.28	$1.28^c \pm 0.28$	1.36 ± 0.30	1.52 ± 0.41
MMI SDS	0.07 ± 0.84	$-0.84^{c,f} \pm 1.10$	-0.23 ± 0.87	-0.04 ± 0.92
Protein:mineral (kg/kg)	3.27 ± 0.54	$3.51^c \pm 0.53$	3.16 ± 0.39	3.16 ± 0.41

¹ All values are means \pm SDs. BMC, bone mineral content; BMD, bone mineral density; LS BMD, lumbar spine BMD; LS BMAD, LS (L2–L4) bone mineral apparent density, which is a 3-dimensional “volumetric” measurement that is calculated from the 2-dimensional BMD measured by dual-energy X-ray absorptiometry [reference data used to calculate LS BMAD SDSs (30)]; SDS, SD score [calculated from 533 contemporary healthy children (JCK Wells, JE Williams, and MS Fewtrell, unpublished data, 2002–2007)]; FM, fat mass; FMI, FM index (FM divided by the square of height); FFM, fat-free mass; FFMI, FFM index (FFM divided by the square of height); PMI, protein mass index (protein mass divided by the square of height); MMI, mineral mass index (mineral mass divided by the square of height). ^{a,b,c}Comparisons of CF boys and control boys or CF girls and control girls (paired t test): ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$; ^{d,e,f}CF and control children compared with reference data (one-sample t test): ^d $P < 0.05$, ^e $P < 0.01$, ^f $P < 0.001$.

TABLE 3Summary of comparisons (before adjustment for age, height, and puberty)¹

SDS	Boys with CF compared with		Girls with CF compared with	
	Pair match	Reference population	Pair match	Reference population
Weight	NS	NS	↓	↓
Height	NS	↓	↓	↓
BMI	↑	↑	↓	↓
Waist	↑	↑	NS	↑
FMI	NS	NS	↓	↓
FFMI	↑	↑	NS	↓
PMI	NS	NS	NS	NS
MMI	NS	NS	↓	↓

¹ CF, cystic fibrosis; SDS, SD score; FMI, fat mass index (fat mass divided by the square of height); FFMI, fat-free mass index (FFM divided by the square of height); PMI, protein mass index (protein mass divided by the square of height); MMI, mineral mass index (mineral mass divided by the square of height); ↑, denotes that the CF group had significantly higher scores than did the comparison group; ↓, denotes that the CF group had significantly lower scores than did the comparison group. Paired *t* test with pair matches and one-sample *t* test for reference data were used. Reference data were from UK 1990 (14, 15) data for weight, height, BMI, and waist; reference data for body-composition variables were from 533 children measured by the 4-component model (JCK Wells, JE Williams, and MS Fewtrell, unpublished data, 2002–2007).

Protein-to-mineral ratio

In the absence of any expectation that total PM would be abnormal in children with CF, the protein-to-mineral ratio is an indication of the mineralization of lean tissue. The protein-to-mineral ratio is presented in Table 2 with a significantly higher value in girls with CF ($P < 0.01$) than in healthy girls. However, this figure did not reveal whether this result was due to high or low protein or mineral. Comparisons of PMI SDSs indicated no significant difference (mean \pm SD) (boys with CF: 0.36 ± 1.36 , control boys: -0.16 ± 1.11 ; girls with CF: 0.08 ± 1.03 , control girls: -0.05 ± 0.97). However, there was a difference in sexes when MMI SDSs were compared (boys with CF: 0.07 ± 0.84 , control boys: -0.23 ± 0.87 , $P = 0.09$; girls with CF: $-0.84 \pm$

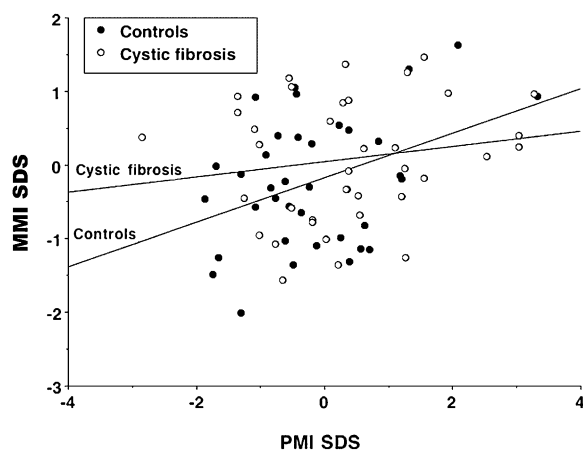


FIGURE 1. Relation between protein mass index (PMI) SD scores (SDS) and mineral mass index (MMI) SDS in boys. PMI (protein mass divided by the square of height) and MMI (mineral mass divided by the square of height) were assessed by the 4-component model of body composition in 37 boys with cystic fibrosis and 37 healthy control boys. Correlations: for cystic fibrosis boys, $r = 0.166$ and $P = 0.325$; for control boys, $r = 0.386$ and $P = 0.018$.

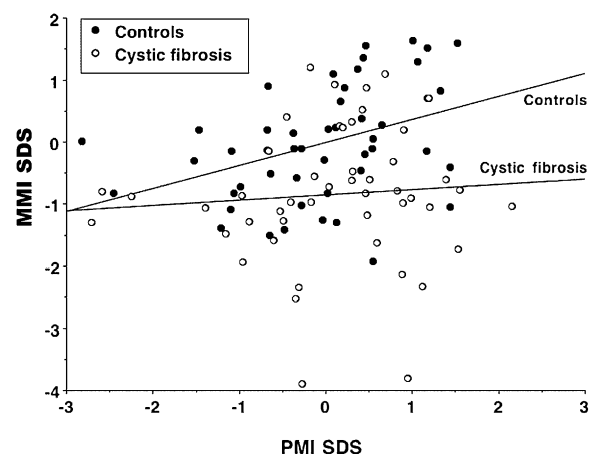


FIGURE 2. Relation between protein mass index (PMI) SD scores (SDS) and mineral mass index (MMI) SDS in girls. PMI (protein mass divided by the square of height) and MMI (mineral mass divided by the square of height) were assessed by the 4-component model of body composition in 48 girls with cystic fibrosis and 48 healthy control girls. Correlations: for cystic fibrosis girls, $r = 0.079$ and $P = 0.596$; for control girls, $r = 0.391$ and $P = 0.006$.

1.10, control girls: -0.04 ± 0.92 , $P = 0.001$). As shown in **Figures 1** (boys) and **2** (girls) for control children, PMI SDSs and MMI SDSs were positively correlated (boys: $r = 0.39$, $P = 0.018$; girls: $r = 0.39$, $P = 0.006$) as expected. However, there was no such correlation in children with CF (boys: $r = 0.17$, $P = 0.325$; girls: $r = 0.08$, $P = 0.596$), and the girls in particular were undermineralized for any given protein values.

Potential confounders that affected body-composition outcomes

Adjusted mean differences in body composition (CF children – control children) before and after adjustment for height and puberty are shown in **Tables 4** (boys) and **5** (girls). Boys with CF had a greater weight ($P < 0.1$) and waist circumference ($P < 0.001$) than did control boys. Girls with CF had lower weight ($P < 0.05$), FM ($P < 0.01$), and hip and midupper arm circumference ($P < 0.01$) than did control girls. An analysis of only prepubertal girls ($n = 24$ pairs) confirmed the deficit in FM. Additional adjustment of MM for bone area (to adjust for bone size in addition to length) did not affect the outcome in either sex. To determine whether differences in body composition were due to different levels of activity, the parent's rating of the child's activity level was added to the model with no effect on the outcome (data not shown).

Relation between body composition and lung function

The mean (\pm SD) FEV₁ percentage predicted for 83 patients (one boy and one girl did not perform spirometry) was $91.3 \pm 20.9\%$ for boys and $77.6 \pm 18.4\%$ for girls ($P < 0.01$). A regression analysis of factors associated with the FEV₁ percentage predicted is presented in **Table 6**. Most indexes of whole-body fat but not subcutaneous fat were significantly positively associated with lung function in girls only ($P < 0.05$). A similar model with either FEV₁ or FEV₁ SDSs indicated a similar pattern. A plot of the FEV₁ percentage predicted and FMI SDSs for girls is shown in **Figure 3** and indicated a positive relation ($r = 0.40$, $P = 0.005$); however, 4 girls had a high FEV₁ percentage predicted with low FMI SDSs.

TABLE 4Differences in size and whole-body composition between boys with cystic fibrosis and control boys ($n = 37$ matched pairs)¹

	Adjusted for age		Adjusted for age and height		Adjusted for age, height, and puberty	
	Mean \pm SEM	<i>P</i>	Mean \pm SEM	<i>P</i>	Mean \pm SEM	<i>P</i>
Weight (kg)	0.7 \pm 1.1	NS	2.0 \pm 0.7	<0.01	2.1 \pm 0.8	<0.01
Height (m) ²	-0.02 \pm 0.01	NS			-0.01 \pm 0.01	NS
BMI (kg/m ²) ²	1.0 \pm 0.4	<0.05			1.1 \pm 0.4	<0.01
TBW (L)	0.1 \pm 0.5	NS	0.7 \pm 0.3	<0.05	0.7 \pm 0.3	<0.05
BV (L)	0.7 \pm 1.1	NS	2.1 \pm 0.8	<0.05	2.1 \pm 0.8	<0.05
FM (kg)	0.7 \pm 0.8	NS	1.3 \pm 0.7	NS	1.3 \pm 0.7	NS
FFM (kg)	-0.1 \pm 0.6	NS	0.7 \pm 0.4	NS	0.7 \pm 0.4	NS
FFM (kg) ³	-0.05 \pm 0.61	NS	0.71 \pm 0.43	NS	0.72 \pm 0.44	NS
FFM hydration (%)	-0.01 \pm 0.5	NS	-0.02 \pm 0.5	NS	-0.04 \pm 0.5	NS
FFM density (kg/L)	0.000 \pm 0.000	NS	0.000 \pm 0.000	NS	0.000 \pm 0.000	NS
PM (kg)	-0.04 \pm 0.2	NS	0.1 \pm 0.2	NS	0.0 \pm 0.2	NS
MM (kg)	-0.02 \pm 0.04	NS	0.03 \pm 0.02	NS	0.03 \pm 0.02	NS
MM (kg) ³	-0.02 \pm 0.04	NS	0.03 \pm 0.02	NS	0.03 \pm 0.02	NS
LS BMAD SDS	0.3 \pm 0.2	NS	0.2 \pm 0.2	NS	0.2 \pm 0.2	NS
Waist circumference (cm)	4.9 \pm 1.0	<0.001	5.8 \pm 0.9	<0.001	5.8 \pm 0.9	<0.001
Hip circumference (cm)	-0.2 \pm 1.2	NS	1.2 \pm 0.9	NS	1.2 \pm 0.9	NS
MUAC (cm)	-0.1 \pm 0.5	NS	0.3 \pm 0.5	NS	0.3 \pm 0.5	NS
Log ₄ skinfold thickness	0.04 \pm 0.1	NS	0.1 \pm 0.1	NS	0.1 \pm 0.1	NS

¹ TBW, total body water; BV, body volume; FM, fat mass; FFM, fat-free mass; PM, protein mass; MM, mineral mass; LS BMAD SDS, lumbar spine bone mineral apparent density (size-adjusted bone mineral density) SD score; MUAC, midupper arm circumference; Log₄ skinfold thickness, bicep + tricep + subscapular + suprailiac ($n = 36$ pairs). General linear model adjusted for group, matched pairs, and puberty as fixed factors and age and height as continuous variables.

² Adjusted for age and puberty only.

³ Additionally adjusted for lumbar spine bone area.

Comparison between 2CMs and 4CM

A bias (2CM FM - 4CM FM) was calculated for the 4 methods described (Table 7). The mean FM from skinfold-thickness measurements was significantly underestimated by between 3% and 21% depending on sex and whether the children had CF or were healthy. Hydrometry was significantly overestimated by 8–13% in girls only ($P < 0.001$). Values from densitometry underestimated FM in girls, although this was only significant in control girls (8%, $P < 0.001$), and DXA estimation of FM was underestimated in control boys by 7% and overestimated in girls with CF by 3% ($P < 0.05$). Correlation analyses indicated a significant relation between the magnitude of the bias and the magnitude of FM when using skinfold-thickness measurements for all control children and girls with CF and when using DXA in control girls.

DISCUSSION

In this study we 1) compared the body composition of children with CF to healthy children by using case-control and reference populations 2), investigated associations between body composition and lung function in children with CF, and 3) compared outcomes obtained by using the gold-standard 4CM to those from more commonly used 2CM techniques for assessing body composition.

Body composition

Suboptimal growth in patients with CF affects morbidity and mortality with stunting (33) and wasting (34) as independent

predictors of survival. Deficits in body composition are most severe in older patients with reduced bone, LM, and FM (35, 36). Even with normal BMI, FFM and BMD depletion has been noted in adults (37, 38) and children and adolescents (39) with CF. Low weight for height in adults with CF occurs particularly in women (40), who also die at a younger age than men (41). A previous study showed that the greatest decline in nutritional status occurs after the onset of puberty, particularly in girls (42), although other studies suggest that deterioration in growth and nutritional status occurs throughout childhood (4, 43, 44). There is a paucity of body-composition studies in children with CF, and comparison is difficult because of different methodologies used, sexual dimorphism, age ranges, and whether appropriate size adjustments have been applied. However, several recent studies that used DXA noted a reduction of FM and/or FFM (4, 45, 46), and Stettler (47) noted a reduction in both FFM by hydrometry and FM by total-body electrical conductivity in boys only, whereas Ahmed et al (48), who used skinfold-thickness measurements, suggested that although both sexes have reduced FM, a reduction of FFM occurs in older boys. To our knowledge, no previous study has used a gold-standard technique to assess body composition in children with CF.

Our study identified clear sex differences in body composition in young patients, which indicated that abnormalities in female patients may be established much earlier than previously considered (42). Although boys with CF had body composition similar to healthy control subjects, girls had a deficit of FM. It is possible that the method we used to categorize pubertal development may not have been sensitive to early hormonal changes; however, the deficit was seen in prepubertal girls. In



TABLE 5Differences in size and whole-body composition between girls with cystic fibrosis and control girls ($n = 48$ matched pairs)¹

	Adjusted for age		Adjusted for age and height		Adjusted for age, height, and puberty	
	Mean \pm SEM	<i>P</i>	Mean \pm SEM	<i>P</i>	Mean \pm SEM	<i>P</i>
Weight (kg)	-5.7 \pm 1.3	<0.001	-2.0 \pm 1.0	NS	-2.0 \pm 0.9	<0.05
Height (m) ²	-0.05 \pm 0.01	<0.001			-0.05 \pm 0.01	<0.001
BMI (kg/m ²) ²	-1.8 \pm 0.5	<0.001			-1.4 \pm 0.4	<0.001
TBW (L)	-1.9 \pm 0.6	<0.001	-0.02 \pm 0.3	NS	-0.04 \pm 0.3	NS
BV (L)	-5.9 \pm 1.3	<0.001	-2.2 \pm 1.1	NS	-2.2 \pm 0.9	<0.05
FM (kg)	-3.4 \pm 0.8	<0.001	-2.1 \pm 0.8	<0.01	-2.1 \pm 0.7	<0.01
FFM (kg)	-2.2 \pm 0.9	<0.05	0.3 \pm 0.5	NS	0.3 \pm 0.4	NS
FFM (kg) ³	-2.46 \pm 0.73	<0.001	0.03 \pm 0.40	NS	0.02 \pm 0.37	NS
FFM hydration (%)	-0.3 \pm 0.4	NS	-0.4 \pm 0.5	NS	-0.4 \pm 0.5	NS
FFM density (kg/L)	-0.001 \pm 0.000	NS	0.000 \pm 0.000	NS	0.000 \pm 0.000	NS
PM (kg)	-0.3 \pm 0.2	NS	0.2 \pm 0.1	NS	0.2 \pm 0.1	NS
MM (kg)	-0.3 \pm 0.1	<0.001	-0.1 \pm 0.0	NS	-0.1 \pm 0.0	NS
MM (kg) ³	-0.25 \pm 0.06	<0.001	-0.07 \pm 0.04	NS	-0.07 \pm 0.04	NS
LS BMAD SDS	-0.2 \pm 0.3	NS	0.1 \pm 0.3	NS	0.1 \pm 0.3	NS
Waist circumference (cm)	-2.3 \pm 1.1	<0.05	-0.3 \pm 1.1	NS	-0.4 \pm 0.9	NS
Hip circumference (cm)	-6.4 \pm 1.3	<0.001	-3.0 \pm 1.1	<0.05	-3.0 \pm 1.0	<0.01
MUAC (cm)	-2.6 \pm 0.6	<0.001	-1.4 \pm 0.5	<0.05	-1.4 \pm 0.5	<0.01
Log ₄ skinfold thicknesses	-0.2 \pm 0.1	<0.01	-0.1 \pm 0.1	NS	-0.2 \pm 0.1	NS

¹ TBW, total body water; BV, body volume; FM, fat mass; FFM, fat-free mass; PM, protein mass; MM, mineral mass; LS BMAD SDS, lumbar spine bone mineral apparent density (size-adjusted bone mineral density) SD score; MUAC, midupper arm circumference; Log₄ skinfold thickness, bicep + tricep + subscapular + suprailiac ($n = 45$ pairs). General linear model adjusted for group, matched pairs, and puberty as fixed factors and age and height as continuous variables.

² Adjusted for age and puberty only.

³ Additionally adjusted for lumbar spine bone area.

accordance with previous research, children with CF in this study were shorter than healthy children, but surprisingly, boys had higher BMI SDSs than did healthy control boys and the reference population. This was mainly due to 4 boys (boys *a* through *d*) with BMI SDSs >1.64 (95th percentile). BMI and FMI SDSs for these boys were 1.71 and 1.49 (*a*), 1.83 and 1.49 (*b*), 1.96 and

0.32 (*c*), and 2.42 and 1.99 (*d*), which indicated that high BMI SDSs do not always reflect excess fat. Simple skinfold-thickness measurements identified boy *c* as having less subcutaneous fat than the other 3 boys. It may be prudent to identify the nature of high BMI in these patients and implement dietary interventions to avoid the complications of excess fat in adult life. Girls with

TABLE 6Simple regression analysis of factors associated with forced expired volume in 1 s percentage predicted¹

	Boys ($n = 37$)					Girls ($n = 48$)				
	<i>B</i>	SEM	<i>t</i>	<i>P</i>	<i>r</i> ²	<i>B</i>	SEM	<i>t</i>	<i>P</i>	<i>r</i> ²
BMI SDS	5.31	3.68	1.44	0.159	0.057	5.91	2.73	2.16	0.036	0.094
4C FM (kg)	1.25	1.25	1.01	0.321	0.030	3.05	1.11	2.76	0.008	0.151
Log sum of 4 skinfold thicknesses ²	7.63	9.16	0.83	0.411	0.021	13.6	8.15	1.67	0.103	0.063
DXA FM (kg)	0.15	1.34	0.11	0.910	1.001	2.25	1.14	1.96	0.056	0.084
Hydrometry FM (kg)	1.24	1.19	1.04	0.307	0.032	2.63	1.14	2.31	0.025	0.112
Densitometry FM (kg)	1.39	1.25	1.11	0.276	0.039	3.04	0.96	3.19	0.003	0.191
4C FFM (kg)	-0.25	2.26	-0.11	0.911	0.001	1.72	1.53	1.12	0.268	0.032
DXA FFM (kg) ³	3.58	2.29	1.57	0.126	0.070	2.05	1.42	1.45	0.156	0.049
Hydrometry FFM (kg)	-0.42	2.13	-0.20	0.846	0.001	0.94	1.46	0.64	0.525	0.013
Densitometry FFM (kg)	-0.48	2.12	-0.23	0.823	0.004	-1.42	1.74	-0.82	0.417	0.019
4C MM (kg) ⁴	8.21	31.7	0.26	0.797	0.003	23.7	23.3	1.02	0.316	0.028
DXA BMC (kg) ⁴	10.9	38.5	0.30	0.788	0.003	47.7	28.8	1.66	0.105	0.065
DXA BMD (g/m ³)	-47.4	75.6	-0.06	0.535	0.012	58.3	66.8	0.87	0.387	0.021

¹ SDS, SD score; FM, fat mass; FFM, fat-free mass; 4C, 4-component; DXA, dual-energy X-ray absorptiometry; MM, total body mineral mass; BMC, bone mineral content; BMD, bone mineral density. Factors, except BMI SDS, were adjusted for height. Each line represents a new model (a separate analysis).

² $n = 36$ boys and 45 girls.

³ DXA lean + bone mineral.

⁴ Also adjusted for lumbar spine bone area.



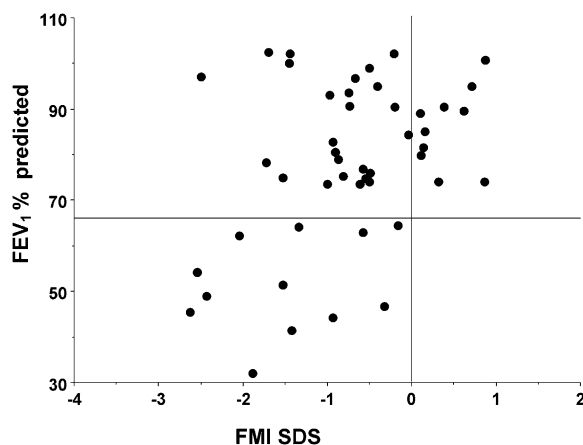


FIGURE 3. Relation between percentage predicted forced expired volume in 1 s (FEV₁% predicted) and fat mass index (FMI) SD scores (SDS) in girls with cystic fibrosis. FMI (FM divided by the square of height) SDS were assessed by the 4-component model of body composition. Correlation in 47 girls with cystic fibrosis: $r = 0.40$ and $P = 0.005$.

CF were smaller overall than control girls, which was perhaps related to the slightly higher than expected weights of control girls and the delayed puberty and suboptimal health (indicated by poorer lung function) of girls with CF.

In healthy individuals, including the healthy children in this study, proteins and minerals were positively correlated. However, children with CF did not exhibit this relation; the range of MMI SDSs for boys with CF was similar to that of control subjects, but the range for girls was much wider and tended toward the lower values. Girls with CF showed undermineralization for given protein values. This suggests that some aspect of the disease affects the protein-to-mineral relation, particularly in girls, and it is possible that this represents a direct affect of the *CFTR* gene on bone metabolism. More research is needed to see if the alteration in the ratio becomes more pronounced in the boys with age and whether the difference persists over time.

We previously reported normal BMD and BMAD in thirty-two 8–12-y-old children with CF (49); although boys in the current study had normal BMD and BMAD values, the values for girls were significantly lower. The 24 prepubertal girls had normal bone BMAD but low BMD ($P < 0.001$), which suggested that low BMD reflected small size and delayed puberty.

Differences when data from patients were compared with data from either matched control children or a large reference population are summarized in Table 3, and these highlight some of the difficulties of comparing studies of different designs. In our study, boys were identified as shorter than the reference population but not by a case-control analysis, whereas girls had significantly lower FFM SDSs than did the reference population but not compared with matched control subjects. The methods of matching and analysis must be considered when studies are compared.

Lung function

There is evidence that improving nutrition can delay deterioration in lung function and improve survival (50, 51), but deficits of bone mass, LM, and FM in young patients with mild lung disease and normal nutritional status have been reported (47, 52). A consistent positive relation between FFM and lung

function has been noted in adults (36, 38) and children (45, 53, 54). However, not all studies separated the sexes or size-adjusted components of body composition. We chose FEV₁ as an outcome measure because it is a good surrogate marker of clinical status and predicts mortality and showed clear sex differences in the relation between FEV₁ and body composition. Contrary to previous research, FFM was not associated with the FEV₁ percentage predicted in either boys or girls, but we identified a significant positive relation with FM adjusted for height in girls with CF. This discordance with previous studies may be because of the methodologies used (in our study the relation was not identified by skinfold-thickness measurements), the narrow age range of young children studied, the adjustment of FM for stature, or the analysis of sexes separately. The sex differences may reflect differences in body composition during growth, with relatively greater gains in FFM in boys and FM in girls. It is likely that the positive association between FM and lung function in girls is a reflection of their poor nutritional status because of the generally low FM in this group. However, some girls with low FM had good lung function (Figure 3); of the 4 girls with FMI SDSs less than -1.5 and FEV₁ $>78\%$, 3 girls were more physically active than their peer group, and one girl was as physically active as her peer group. This finding warrants further investigation and suggests that nutritional and physical therapies need to be sex specific. We could not analyze our data by genotype and sex because of low numbers in each group.

Methodology

The findings of previous body-composition studies were influenced by the methodology used. Many studies used height and weight measurements to assess growth, but these measurements do not provide information about body composition, and many 2CM techniques used in previous studies, including skinfold-thickness measurements, bio-electrical impedance, hydrometry, and densitometry, merely predicted FM and FFM. The assumptions used (derived from healthy populations) about the nature of the FFM are likely to be less valid for patients. More recent studies used DXA to quantify BMC, FM, and FFM; however, soft tissue assessments were biased in some individuals (6, 55), which made comparison between patients and control subjects or comparisons over time (where there is a change in body size) problematic. Our findings, which compared commonly used 2CM techniques with the gold-standard 4CM, illustrated the effect of methodology with a significant bias (depending on sex and health status) compared with 4CM for all techniques under investigation. In addition, limits of agreement for 2CMs varied from ± 2 to 3 kg for skinfold-thickness measurements to ± 1 to 2 kg for other methods, which indicated that the accuracy is poor in individuals. All 2CM techniques, apart from skinfold-thickness measurements, were consistent with the criterion method in finding FM related to the FEV₁ percentage predicted in girls only. However, the comparison of body composition between children with CF and control children highlighted different findings with boys having more FFM by hydrometry and skinfold-thickness measurements. This is a possible explanation for the contradictory findings of previous studies.

In the current study, a positive significant relation between FM and FEV₁ percentage predicted in girls was identified by using



TABLE 7

Analysis of mean bias in fat mass measured by 2-component models compared with the 4-component model¹

	<i>n</i>	Absolute bias ²	Absolute 95% limits of agreement	Bias as a percentage of the mean ³	<i>P</i> for the bias from zero ⁴	Correlation bias/mean <i>r</i> ⁵	<i>P</i> for correlation
		kg	kg	%			
Skinfold thickness ⁶							
CF boys	37	−0.75	±2.90	−13.2	<0.01	−0.07	NS
CF girls	48	−0.90	±2.10	−15.7	<0.001	−0.36	<0.05
Control boys	37	−1.00	±1.97	−18.6	<0.001	−0.62	<0.001
Control girls	47	−1.65	±2.52	−21.2	<0.001	−0.50	<0.001
Skinfold thickness ⁷							
CF boys	36	−0.64	±2.94	−5.13	<0.05	−0.78	<0.001
CF girls	45	−0.43	±1.30	−3.56	<0.05	−0.66	<0.001
Control boys	37	0.30	±2.42	−10.1	<0.001	−0.79	<0.001
Control girls	46	−1.35	±2.98	−13.8	<0.001	−0.82	<0.001
Hydrometry							
CF boys	37	0.16	±2.27	4.24	NS	−0.17	NS
CF girls	48	0.70	±1.31	13.1	<0.001	−0.03	NS
Control boys	37	0.30	±2.43	3.84	NS	−0.22	NS
Control girls	48	0.59	±1.01	7.98	<0.001	−0.26	NS
DXA							
CF boys	37	−0.25	±1.93	−4.97	NS	−0.05	NS
CF girls	48	0.27	±1.50	2.97	<0.05	0.23	NS
Control boys	37	−0.29	±1.38	−6.89	<0.05	0.13	NS
Control girls	48	0.19	±1.77	0.34	NS	0.43	<0.01
Densitometry							
CF boys	37	0.03	±1.25	−0.36	NS	0.14	NS
CF girls	48	−0.21	±1.91	−5.92	NS	0.11	NS
Control boys	37	0.05	±1.53	0.05	NS	0.15	NS
Control girls	48	−0.50	±1.24	−7.72	<0.001	0.14	NS

¹ CF, cystic fibrosis; DXA, dual-energy X-ray absorptiometry. Analyses were conducted by using the method of Bland and Altman (31).² The group mean value measured by 2-component models minus the group mean value measured by the 4-component model.³ The bias of the natural log values × 100.⁴ Paired *t* test.⁵ Pearson's correlation between the bias and mean values in individuals.⁶ Calculated by using the age- and sex-specific equations of Slaughter et al (19).⁷ Calculated by using the age- and sex-specific equations of Deurenberg et al (20).

4CM, hydrometry, and densitometry, and FM assessed by using DXA approached significance. Some studies that used DXA used LM (ie, tissue that is not fat or bone) and reported the LM as FFM (usually defined as any tissue that is not fat). Therefore, we repeated the analyses by using LM from DXA, and still there was no significant relation in either sex. In addition, with the exception of King et al (38), most studies did not size adjust FM and FFM when investigating the relation with the FEV₁ percentage predicted. Therefore, we repeated our analyses without height adjustment and showed that no components were significantly related to the FEV₁ percentage predicted. This suggested that differences in findings between studies may be, in some part, due to whether height adjustments were made.

All children in this study performed ≥2 measurement procedures to improve accuracy (10), but children with CF generally needed to undergo more ADP measurements to ensure consistency. It is likely that much of the variation was due to erratic breathing patterns, and therefore, single ADP measurements in this group may have been inaccurate.

Limitations

We used predicted rather than measured lung volume when we calculated BV by ADP because of the difficulty of performing the

technique for young children. However, a study of children with CF that derived FFM from ADP concluded that there was no significant difference in FFM calculations between measured or predicted lung volumes (56).

Because exercise affects the amount of lean tissue, we repeated the analyses and took into account parental reported exercise levels, and there were no differences in outcomes. However, parental ratings may have been affected by differing expectations in healthy and chronically ill patients, and this needs to be addressed in future research.

Conclusions

To our knowledge, this was the first study to use a gold-standard method to measure body composition in children with CF. We observed clear sex differences, even in prepubertal children, and because of the poorer prognosis in girls, these differences warrant further investigation. Four boys with CF had BMI SDSs in the obese range, and in 3 cases, this was due to excess fat. It would seem prudent to carefully monitor children with high BMI to avoid future health problems because of excess fat. Finally, our results highlight some of the potential methodologic explanations behind inconsistent findings in previous

studies. In particular, 2CMs should be used with caution and always adjusted for age, height, and sex.

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The authors' responsibilities were as follows—JKCW, MSF, AJ, CB and RS: conceived the study; JEW and CMW: measured subjects; JEW: modeled body-composition data and wrote the first draft of the manuscript; JEW, JCKW, and MSF: conducted statistical analyses; and all authors: contributed to the revision of the manuscript. None of the authors had a conflict of interest.

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Body-composition reference data for simple and reference techniques and a 4-component model: a new UK reference child^{1–3}

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ABSTRACT

Background: A routine pediatric clinical assessment of body composition is increasingly recommended but has long been hampered by the following 2 factors: a lack of appropriate techniques and a lack of reference data with which to interpret individual measurements. Several techniques have become available, but reference data are needed.

Objective: We aimed to provide body-composition reference data for use in clinical practice and research.

Design: Body composition was measured by using a gold standard 4-component model, along with various widely used reference and bedside methods, in a large, representative sample of British children aged from 4 to ≥ 20 y. Measurements were made of anthropometric variables (weight, height, 4 skinfold thicknesses, and waist girth), dual-energy X-ray absorptiometry, body density, bioelectrical impedance, and total body water, and 4-component fat and fat-free masses were calculated. Reference charts and SD scores (SDSs) were constructed for each outcome by using the lambda-mu-sigma method. The same outcomes were generated for the fat-free mass index and fat mass index.

Results: Body-composition growth charts and SDSs for 5–20 y were based on a final sample of 533 individuals. Correlations between SDSs by using different techniques were ≥ 0.68 for adiposity outcomes and ≥ 0.80 for fat-free mass outcomes.

Conclusions: These comprehensive reference data for pediatric body composition can be used across a variety of techniques. Together with advances in measurement technologies, the data should greatly enhance the ability of clinicians to assess and monitor body composition in routine clinical practice and should facilitate the use of body-composition measurements in research studies. *Am J Clin Nutr* doi: 10.3945/ajcn.112.036970.

INTRODUCTION

Growth charts for weight and height have been the backbone of pediatric clinical assessment of nutritional status for decades (1–4). However, efforts to obtain more detailed information on body composition have long been hampered by 2 challenges. First, methods for the measurement of pediatric body composition have taken time to develop. Only within the past decade have techniques such as dual-energy X-ray absorptiometry (DXA)⁴, air-displacement plethysmography, bioelectrical impedance analysis (BIA), and isotope dilution become widely applied in the pediatric population (5). Second, even when such techniques are available, interpretation is severely hindered by the lack of appropriate reference data.

Thus, clinical practice has been strongly influenced by the nature of available data. Reference data for British children's skinfold-thickness measurements were provided in the 1970s (1). More recently, reference data for UK children's BMI were published in the 1990s (6) by using Cole's lambda-mu-sigma (LMS) method to take into account age changes in the variability and skewness of data (7). These BMI charts have become the primary UK reference for the interpretation of nutritional status in the clinic and have been replicated in many other populations (8–11). To aid convergence between these approaches, skinfold-thickness data were also converted to the LMS format (12).

International BMI cutoffs for categorizing overweight and obesity and underweight have also been published (13, 14). Such BMI data have been widely adopted, in part because of their value in predicting clinical outcomes. Nevertheless, the data suffer from limitations when more-detailed information about fat mass or fat-free mass is required. Historically, fat-free tissue has been considered the functional and dynamic component of weight, with fat mass conceptualized as a relatively inert energy store. Recent studies that identified numerous hormonal products of adipose tissue have challenged this view, and adipose tissue is understood to play a complex regulatory role and exerts many of its effects on fat-free tissue (15). Therefore, there is increasing interest in the ability to categorize fat-free mass and fat mass and monitor their changes over time.

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⁴ Abbreviations used: BIA, bioelectrical impedance analysis; DXA, dual-energy X-ray absorptiometry; LMS, lambda-mu-sigma; SDS, SD score; TBW, total body water; 4C, 4-component.

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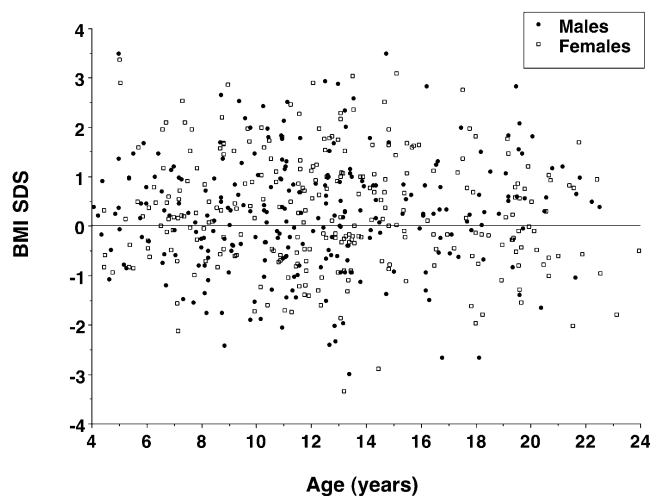


FIGURE 1. Distribution of BMI SDS against age in the sample. $n = 261$ males and 272 females. SDS, SD score.

We previously summarized a number of contexts in which information about body composition could be of value to the pediatrician (16) and also described the methodologies available (5). However, until reference data for children's body composition are available, measurements of individual patients will remain difficult to interpret (17). Reference data for individual techniques (eg, skinfold thicknesses, BIA, and DXA), have been reported in the literature (18–25), but to our knowledge, no study has provided comprehensive reference data on a range of techniques in any single population. In this article, we describe reference data for a number of different measures of body composition, which will allow our reference data set to be used across a variety of techniques.

SUBJECT AND METHODS

A total of 565 normal healthy children, adolescents, and young adults aged 4–23 y were recruited by using flyers and newspaper adverts in London and the southeast of England starting in 2001. There were no exclusion criteria for BMI, and thus, some individuals were categorized as overweight or obese, but they were not recruited directly from obesity weight-loss clinics and had no disease that might have adversely affected growth and development. The lower age limit

of 4 y was chosen on the basis of our previous work because younger children are unlikely to satisfy the protocol for air-displacement plethysmography. Data collection was extended to young adults to cover the entire pediatric age range. Ethical approval was granted by the Ethical Committee of University College London Institute of Child Health and Great Ormond Street Hospital. All individuals attended our body-composition investigation suite located at Great Ormond Street Hospital for a 2-h measurement session.

Weight and height were measured by using standard protocols. Body weight was measured in duplicate as part of the air-displacement plethysmography protocol. Height was measured by using a wall-mounted stadiometer (Holtain). BMI (in kg/m^2) was calculated as weight divided by the square of height. Data on weight, height, and BMI were converted to SD score (SDS) format by using UK reference data (6, 26). Obesity was defined as BMI >95th percentile (SDS >1.64), and overweight was defined as BMI >85th percentile (SDS >1.04) (6). Pubertal development was assessed by using Tanner staging with self-assessment based on line drawings.

Skinfold-thickness measurements were performed in triplicate at the biceps, triceps, subscapular, and suprailiac sites, and the mean of the 3 values was used. Waist girth was measured by using a nonstretchable fiberglass tape. BIA was conducted with Tanita BC418MA instrumentation (Tanita Corp); however, this instrument was available only from 2004 onward, and thus, the sample size was 451 (83% of the total) for this outcome. With the use of whole-body values for impedance (Z , in Ω) at 50 kHz, the impedance index [height^2/Z (cm^2/Ω)] was calculated. Numerous pediatric equations have been published for BIA that have left users uncertain as to which equation to select for any given population. Therefore, the BIA output was analyzed in raw units (in cm^2/Ω) to avoid influencing this outcome by the choice of one or another equation. This approach prevented the use of BIA data as an index of adiposity, and therefore, skinfold thicknesses were the primary bedside approach tested for adiposity. Because absolute body-composition values obtained by using predictive equations in combination with such bedside techniques have high SEEs, our combined skinfold thicknesses plus BIA bedside approach had the added advantage that fat-free and fat tissues were assessed by using independent techniques, and error on the adiposity index should have been independent from error on the fat-free mass outcome.

TABLE 1

Summary statistics for anthropometric measures and weight status by sex¹

	M ($n = 261$)	F ($n = 272$)
Weight SDS	0.31 ± 1.08 (−2.42 to 3.44) ²	0.42 ± 1.10 (−2.75 to 3.46)
Height SDS	0.21 ± 0.96 (−2.09 to 3.28)	0.34 ± 1.02 (−2.77 to 3.42)
BMI SDS	0.24 ± 1.15 (−2.99 to 3.49)	0.31 ± 1.15 (−3.33 to 3.32)
Percentage of fat (4C model) ³	19.2 ± 8.0 (4.9–45.5)	27.0 ± 8.0 (11.7–46.9)
Prevalence (%)		
Overweight	9.2	10.7
Obese	11.5	14.7

¹ SDSs were calculated by using UK reference data (6, 25). Overweight was categorized as BMI SDS >1.04 (85th percentile). Obese was categorized as BMI SDS >1.64 (95th percentile). SDS, SD score; 4C, 4-component.

² Mean \pm SD; range in parentheses (all such values).

³ The difference in the percentage of fat between sexes was determined by using multiple regression analysis to assess the significance of female sex with adjustment for age, $P < 0.0001$.

Measurements of total body water (TBW; in L) by using deuterium dilution, bone mineral content (in kg) by using DXA (Lunar Prodigy, software version 6.7; GE Medical Systems), and body volume (in L) in duplicate by using air-displacement plethysmography (Bodpod; Life Measurements) were obtained as described previously, with postdose saliva collected after 4–5 h (27). The deuterium-dilution space was converted to TBW with the assumption that the degree of overestimation that was attributable to proton exchange was 1.044 (28). Lung volume was predicted rather than measured in plethysmography measurements because we have shown that a large proportion of children are unable to complete the lung-volume measurement protocol satisfactorily. Values for fat-free mass by using DXA were the sum of lean soft tissue and bone mineral mass.

The 4-component (4C) model is considered the most accurate in vivo approach for the differentiation of fat and fat-free masses and is particularly valuable in patients in whom assumptions of constant fat-free tissue composition are not valid (29, 30). The 4C model used in this study to calculate lean mass and fat mass (FM) has been described previously (31, 32) and uses the following equations:

$$\text{FM} = (2.747 \times \text{BV}) - (0.710 \times \text{TBW}) + (1.460 \times \text{BMC}) - (2.050 \times \text{WT}) \quad (1)$$

$$\text{FFM} = \text{WT} - \text{FM} \quad (2)$$

where FM is fat mass, BV is body volume, BMC is bone mineral content, FFM is fat-free mass, and WT is body weight. The proportion of fat in weight (percentage of fat) was calculated as

$$(\text{FM} \div \text{WT}) \times 100 \quad (3)$$

In our laboratory, the precision is 1% for TBW (32) and 0.24 L for BV (33). The precision of BMC is 1.1% (34).

Statistics

Analyses were conducted for a range of adiposity and fat-free mass outcomes. For adiposity, the outcomes were 1) each of the 4 skinfold thicknesses, 2) waist girth, 3) whole-body, arm, leg, and trunk fat mass from DXA, 4) body density by air-displacement plethysmography, and 5) 4C fat mass. For fat-free mass, the outcomes were 1) TBW, 2) height^2/Z , 3) whole-body, arm, leg, and trunk fat-free mass by using DXA, and 4) 4C fat-free mass. For the 4C data, the fat-free mass index ($\text{fat-free mass}/\text{height}^2$) and fat mass index ($\text{fat mass}/\text{height}^2$) were also calculated, as described and recommended previously (17, 35). Each of these variables was converted to an SDS.

Sex-specific values by month of age were obtained for all body-composition outcomes by using the LMS method (LMS Chart Maker; Medical Research Council) (7). This statistical approach, which has been widely used to construct reference data for traits that incorporate the effects of growth, provides the following 3 outputs: 1) a smoothed median (M or μ) curve, which represents how the outcome varies in relation to age, 2) the CV (S or σ), which models the scatter of values around

the mean and adjusts for any nonuniform dispersion, and 3) the skewness (L or λ), which is addressed by using age-specific Box-Cox transformation to achieve a normal distribution. Adiposity indexes were fitted by using original age, and fat-free mass outcomes were fitted by using rescaled age, which improved the goodness of fit for monotonic data by fitting the M curve twice. The goodness of fit was assessed by using the Bayesian Information Criterion, with the addition of an extra unit of complexity to the model only if it reduced the deviance by more than $\log_e(N)$ units, where N was the sample size. As the precision of the M curve at any age depends on data points at younger and older ages, precision is lower at the extremes of the age range. Therefore, we fitted the data for all ages (4–23 y) and derived LMS values for the age range 5–20 y.

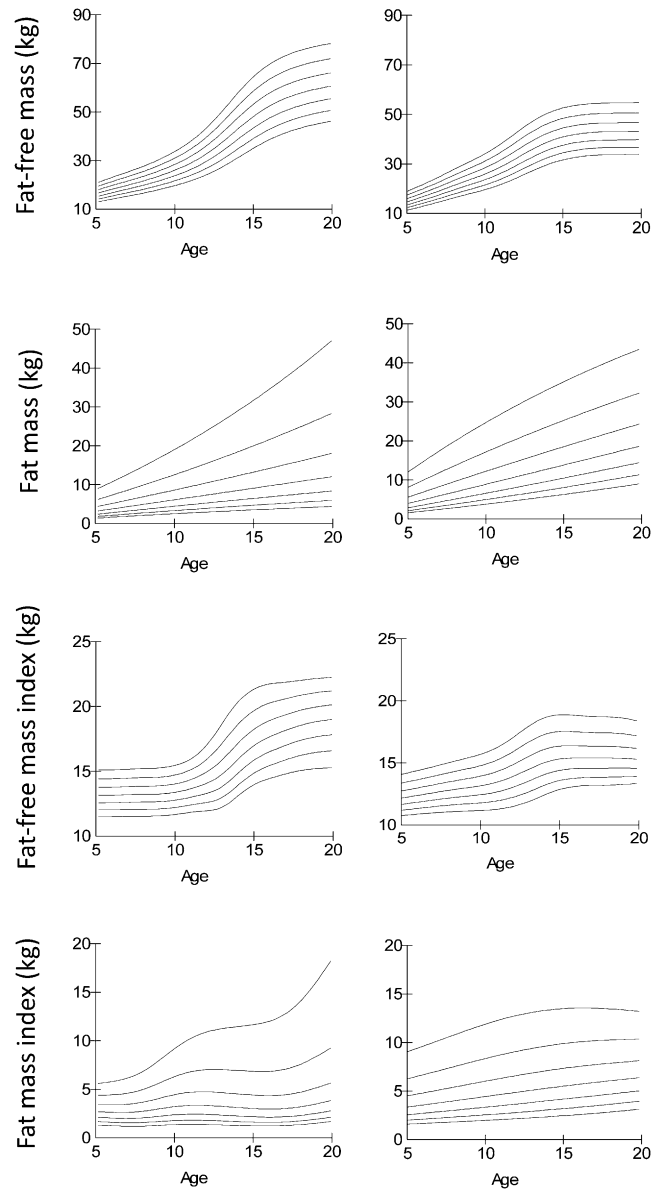


FIGURE 2. Percentiles of fat-free mass, fat mass, fat-free mass index, and fat mass index by the 4-component model for males ($n = 261$; left panels) and females ($n = 272$; right panels). The second, ninth, 25th, 50th, 75th, 91st, and 98th percentiles are displayed in ascending order.

TABLE 2Fat-free mass reference data for males and females by z score or percentile¹

Age	Males							Females						
	$z = -2.0$	$z = -1.33$	$z = -0.67$	$z = 0$	$z = 0.67$	$z = 1.33$	$z = 2.0$	$z = -2.0$	$z = -1.33$	$z = -0.67$	$z = 0$	$z = 0.67$	$z = 1.33$	$z = 2.0$
5.0 y	12.76	13.89	15.08	16.35	17.70	19.13	20.64	11.24	12.27	13.39	14.60	15.91	17.33	18.86
6.0 y	14.10	15.37	16.72	18.16	19.69	21.31	23.03	12.83	14.02	15.30	16.70	18.21	19.85	21.62
7.0 y	15.32	16.73	18.23	19.83	21.54	23.34	25.26	14.49	15.84	17.31	18.91	20.64	22.51	24.55
8.0 y	16.56	18.12	19.79	21.56	23.45	25.45	27.58	16.23	17.77	19.45	21.26	23.24	25.38	27.71
9.0 y	18.02	19.76	21.62	23.61	25.73	27.98	30.38	17.88	19.61	21.49	23.54	25.77	28.20	30.83
10.0 y	19.66	21.62	23.72	25.98	28.38	30.95	33.68	19.56	21.49	23.60	25.89	28.39	31.12	34.08
11.0 y	21.60	23.85	26.28	28.88	31.67	34.64	37.83	21.62	23.79	26.16	28.74	31.56	34.63	37.98
12.0 y	24.06	26.71	29.56	32.63	35.94	39.48	43.27	24.23	26.66	29.31	32.21	35.37	38.82	42.58
13.0 y	27.24	30.36	33.75	37.41	41.35	45.59	50.14	27.06	29.71	32.61	35.76	39.19	42.93	47.00
14.0 y	31.09	34.72	38.65	42.91	47.50	52.44	57.75	29.67	32.46	35.49	38.77	42.34	46.21	50.41
15.0 y	35.03	39.06	43.41	48.12	53.19	58.64	64.50	31.62	34.46	37.54	40.87	44.48	48.38	52.59
16.0 y	38.55	42.82	47.43	52.39	57.73	63.46	69.59	32.81	35.67	38.76	42.10	45.70	49.59	53.78
17.0 y	41.35	45.74	50.47	55.55	60.99	66.82	73.05	33.46	36.32	39.41	42.75	46.34	50.21	54.38
18.0 y	43.45	47.90	52.68	57.80	63.28	69.13	75.38	33.71	36.57	39.67	43.00	46.58	50.45	54.61
19.0 y	45.02	49.50	54.30	59.43	64.92	70.77	77.00	33.75	36.62	39.71	43.04	46.63	50.49	54.65
20.0 y	46.24	50.73	55.54	60.68	66.17	72.01	78.22	33.86	36.73	39.82	43.15	46.73	50.59	54.75

¹ z -score equivalents in percentiles are as follows: $-2 = 2.3\%$, $-1.33 = 9.2\%$, $-0.67 = 25.2\%$, $0 = 50\%$, $0.67 = 74.8\%$, $1.33 = 90.8\%$, and $2 = 97.7\%$.

These data represent new body-composition growth charts available for both the 4C model and individual techniques such as DXA, TBW, body density, BIA, and skinfold thicknesses. Such charts allow for the monitoring of adiposity and fat-free mass over time to improve the understanding of the effects of disease and treatments. We also calculated values for fat-free mass, fat mass, fat-free mass index, and fat mass index by the 4C model for each sex for the following z -score cutoffs: -2 , -1.67 , -1.33 , 0 , 1.33 , 1.67 , and 2 , which are equivalent to percentiles of 2.3%, 9.2%, 25.2%, 50%, 74.8%, 90.8%, and 97.7% respectively.

With the use of this statistical approach, all data were converted to an SDS format, and subsequent analyses on subjects aged 5–20 y were undertaken by using Datadesk 6.1 software (Data Description Inc). The mean of the 4 individual skinfold-

thickness SDSs was also calculated. We quantified the agreement between the individual SDS with the reference 4C SDS. If growth charts are to be adopted by clinicians, they will need to know whether rankings from one technique (eg, skinfold thicknesses) are consistent with ranking by another (eg, DXA or the 4C model). The expression of the data in the SDS format aids such a comparison because, first, it enables the chart rankings to be compared rather than the raw data and, second, because body-composition techniques produce outputs in different units (eg, kg for DXA, kg/m³ for density, mm for skinfold thicknesses, and cm²/Ω for BIA), preventing direct comparisons.

Correlation coefficients were calculated for all of the adiposity and fat-free mass outcomes in each sex. Pearson's correlation coefficients were calculated on the assumption that associations

TABLE 3Fat mass reference data for males and females by z score or percentile¹

Age	Males							Females						
	$z = -2.0$	$z = -1.33$	$z = -0.67$	$z = 0$	$z = 0.67$	$z = 1.33$	$z = 2.0$	$z = -2.0$	$z = -1.33$	$z = -0.67$	$z = 0$	$z = 0.67$	$z = 1.33$	$z = 2.0$
5.0 y	1.37	1.77	2.32	3.11	4.26	6.00	8.72	1.60	2.12	2.87	3.97	5.59	8.09	12.06
6.0 y	1.61	2.09	2.76	3.71	5.11	7.25	10.62	2.02	2.67	3.60	4.95	6.95	9.99	14.78
7.0 y	1.85	2.40	3.18	4.31	5.97	8.52	12.60	2.45	3.23	4.34	5.94	8.29	11.85	17.41
8.0 y	2.08	2.71	3.61	4.91	6.84	9.84	14.66	2.89	3.80	5.09	6.92	9.61	13.66	19.94
9.0 y	2.30	3.01	4.03	5.50	7.72	11.18	16.81	3.34	4.38	5.84	7.91	10.93	15.44	22.37
10.0 y	2.52	3.31	4.44	6.10	8.61	12.56	19.04	3.81	4.97	6.59	8.89	12.23	17.17	24.72
11.0 y	2.73	3.60	4.85	6.70	9.51	13.97	21.37	4.28	5.57	7.36	9.88	13.51	18.86	26.97
12.0 y	2.93	3.89	5.26	7.30	10.42	15.42	23.80	4.76	6.18	8.13	10.86	14.78	20.52	29.15
13.0 y	3.13	4.16	5.66	7.89	11.34	16.90	26.33	5.26	6.80	8.91	11.85	16.04	22.14	31.23
14.0 y	3.32	4.44	6.06	8.49	12.28	18.43	28.96	5.77	7.43	9.69	12.84	17.29	23.72	33.24
15.0 y	3.51	4.71	6.45	9.09	13.22	19.99	31.70	6.29	8.07	10.48	13.82	18.52	25.26	35.17
16.0 y	3.70	4.97	6.84	9.69	14.18	21.59	34.55	6.82	8.72	11.28	14.81	19.74	26.77	37.03
17.0 y	3.87	5.23	7.23	10.28	15.14	23.23	37.53	7.37	9.38	12.09	15.79	20.94	28.24	38.82
18.0 y	4.05	5.48	7.61	10.88	16.12	24.92	40.62	7.93	10.05	12.90	16.78	22.14	29.68	40.53
19.0 y	4.21	5.73	7.99	11.48	17.11	26.64	43.85	8.50	10.74	13.72	17.76	23.32	31.09	42.18
20.0 y	4.38	5.97	8.36	12.08	18.11	28.41	47.21	9.08	11.43	14.55	18.75	24.49	32.46	43.76

¹ z -score equivalents in percentiles are as follows: $-2 = 2.3\%$, $-1.33 = 9.2\%$, $-0.67 = 25.2\%$, $0 = 50\%$, $0.67 = 74.8\%$, $1.33 = 90.8\%$, and $2 = 97.7\%$.

TABLE 4Fat-free mass index reference data for males and females by z score or percentile¹

Age	Males							Females						
	$z = -2.0$	$z = -1.33$	$z = -0.67$	$z = 0$	$z = 0.67$	$z = 1.33$	$z = 2.0$	$z = -2.0$	$z = -1.33$	$z = -0.67$	$z = 0$	$z = 0.67$	$z = 1.33$	$z = 2.0$
5.0 y	11.59	12.13	12.68	13.25	13.84	14.43	15.04	10.75	11.16	11.61	12.11	12.68	13.33	14.07
6.0 y	11.59	12.13	12.69	13.27	13.87	14.49	15.14	10.88	11.32	11.81	12.35	12.94	13.59	14.33
7.0 y	11.50	12.03	12.59	13.17	13.79	14.43	15.11	10.98	11.47	12.00	12.57	13.20	13.89	14.64
8.0 y	11.43	11.95	12.50	13.09	13.72	14.39	15.10	11.05	11.59	12.18	12.81	13.49	14.22	15.03
9.0 y	11.48	11.99	12.55	13.15	13.79	14.49	15.25	11.10	11.69	12.33	13.01	13.76	14.56	15.43
10.0 y	11.61	12.15	12.72	13.35	14.03	14.78	15.59	11.16	11.80	12.49	13.24	14.05	14.93	15.89
11.0 y	11.80	12.38	13.02	13.71	14.48	15.32	16.27	11.31	12.00	12.75	13.56	14.44	15.40	16.45
12.0 y	12.05	12.71	13.44	14.24	15.14	16.15	17.28	11.57	12.29	13.09	13.95	14.91	15.95	17.11
13.0 y	12.45	13.19	14.02	14.95	15.98	17.15	18.49	11.89	12.64	13.47	14.39	15.40	16.52	17.77
14.0 y	12.98	13.81	14.74	15.76	16.91	18.20	19.65	12.24	13.01	13.85	14.80	15.84	17.01	18.33
15.0 y	13.55	14.46	15.46	16.56	17.78	19.13	20.63	12.57	13.33	14.18	15.12	16.18	17.37	18.72
16.0 y	14.07	15.06	16.12	17.28	18.53	19.88	21.36	12.84	13.58	14.41	15.33	16.37	17.56	18.91
17.0 y	14.51	15.57	16.69	17.87	19.13	20.45	21.86	13.03	13.75	14.54	15.43	16.44	17.60	18.93
18.0 y	14.87	15.99	17.15	18.36	19.60	20.88	22.19	13.18	13.85	14.60	15.45	16.41	17.52	18.81
19.0 y	15.14	16.33	17.53	18.74	19.96	21.19	22.42	13.28	13.91	14.61	15.40	16.31	17.37	18.60
20.0 y	15.34	16.60	17.83	19.05	20.24	21.41	22.57	13.35	13.93	14.58	15.32	16.18	17.17	18.35

¹ z -score equivalents in centiles are as follows: $-2 = 2.3\%$, $-1.33 = 9.2\%$, $-0.67 = 25.2\%$, $0 = 50\%$, $0.67 = 74.8\%$, $1.33 = 90.8\%$, and $2 = 97.7\%$

between different z scores were expected to be linear. For central adiposity, we also calculated correlations of SDSs for DXA trunk fat and waist girth. A sex-specific regression analysis was undertaken for the prediction of the 4C fat mass SDS from each individual adiposity SDS, and the 4C fat-free mass SDS from each individual fat-free mass, TBW, or height²/Z SDS. Slopes and intercepts were assessed for the difference from 1 and 0, respectively, and the SEE was calculated. Bland-Altman analysis (36) was used to illustrate the agreement with 4C SDS values for DXA whole-body SDS, BIA SDS, and average skinfold-thickness SDS. A minority of subjects (27 boys and 23 girls; ie, 9.3% of the sample) were of non-European ethnicity; however, this sample size was considered too small to allow ethnic variability in body composition to be addressed.

RESULTS

Valid data were obtained on 533 individuals. Data on 32 other individuals were discarded because either one or more of the basic measurements were unsuccessful ($n = 16$; mostly very young children) or the modeling was unsuccessful ($n = 16$) as indicated by spurious body-composition data. As shown in **Figure 1**, a wide range of BMI SDSs was apparent at all ages. There was no significant correlation between BMI SDS and age in either sex.

Data on anthropometric SDS values and the range of percentage of fat by sex are shown in **Table 1**. On average, our sample was heavier and taller in comparison with the UK reference data of the early 1990s ($P < 0.005$ in all cases). Females unsurprisingly had a significantly greater percentage fat than did

TABLE 5Fat mass index reference data for males and females by z score or percentile¹

Age	Males							Females						
	$z = -2.0$	$z = -1.33$	$z = -0.67$	$z = 0$	$z = 0.67$	$z = 1.33$	$z = 2.0$	$z = -2.0$	$z = -1.33$	$z = -0.67$	$z = 0$	$z = 0.67$	$z = 1.33$	$z = 2.0$
5.0 y	1.41	1.79	2.26	2.84	3.56	4.45	5.53	1.59	1.99	2.53	3.30	4.42	6.14	8.90
6.0 y	1.24	1.60	2.06	2.65	3.38	4.32	5.49	1.67	2.11	2.70	3.54	4.77	6.63	9.58
7.0 y	1.11	1.44	1.88	2.45	3.20	4.17	5.45	1.75	2.22	2.86	3.77	5.10	7.10	10.24
8.0 y	1.12	1.46	1.92	2.54	3.38	4.52	6.10	1.83	2.33	3.03	4.01	5.43	7.56	10.87
9.0 y	1.29	1.69	2.23	2.98	4.03	5.55	7.76	1.91	2.45	3.19	4.24	5.75	8.00	11.47
10.0 y	1.45	1.89	2.50	3.37	4.66	6.59	9.61	1.98	2.56	3.35	4.46	6.06	8.42	12.00
11.0 y	1.51	1.95	2.59	3.52	4.93	7.17	10.91	2.07	2.68	3.51	4.68	6.35	8.79	12.44
12.0 y	1.45	1.88	2.49	3.40	4.83	7.20	11.43	2.16	2.80	3.68	4.90	6.63	9.12	12.80
13.0 y	1.35	1.75	2.32	3.18	4.57	6.96	11.49	2.26	2.93	3.85	5.11	6.88	9.41	13.07
14.0 y	1.28	1.65	2.19	3.02	4.37	6.76	11.54	2.36	3.06	4.01	5.32	7.12	9.65	13.26
15.0 y	1.23	1.59	2.10	2.90	4.23	6.61	11.54	2.47	3.20	4.18	5.51	7.33	9.85	13.38
16.0 y	1.20	1.55	2.06	2.84	4.15	6.54	11.53	2.59	3.34	4.35	5.70	7.52	10.01	13.43
17.0 y	1.22	1.58	2.10	2.90	4.24	6.69	11.84	2.71	3.49	4.51	5.88	7.69	10.13	13.43
18.0 y	1.32	1.70	2.27	3.14	4.60	7.25	12.78	2.84	3.64	4.68	6.05	7.84	10.22	13.38
19.0 y	1.48	1.92	2.56	3.55	5.19	8.17	14.31	2.98	3.79	4.85	6.21	7.98	10.29	13.31
20.0 y	1.67	2.16	2.89	4.01	5.87	9.21	16.01	3.12	3.95	5.01	6.37	8.11	10.35	13.22

¹ z -score equivalents in centiles are as follows: $-2 = 2.3\%$, $-1.33 = 9.2\%$, $-0.67 = 25.2\%$, $0 = 50\%$, $0.67 = 74.8\%$, $1.33 = 90.8\%$, and $2 = 97.7\%$.

TABLE 6Correlations for adiposity outcomes expressed in SDS format[†]

M (<i>n</i> = 245) and F (<i>n</i> = 259)	Biceps	Triceps	Subscapular	Suprailiac	Mean skinfold	Density	4C fat mass	DXA fat mass	DXA arm fat	DXA leg fat	DXA trunk fat
Biceps	—	0.86	0.78	0.76	0.92	−0.79	0.84	0.82	0.87	0.81	0.79
Triceps	0.82	—	0.74	0.82	0.93	−0.82	0.83	0.83	0.86	0.81	0.81
Subscapular	0.68	0.78	—	0.78	0.90	−0.75	0.81	0.84	0.82	0.81	0.83
Suprailiac	0.69	0.80	0.79	—	0.92	−0.82	0.83	0.84	0.83	0.81	0.85
Mean skinfold	0.88	0.94	0.90	0.91	—	−0.87	0.90	0.91	0.92	0.88	0.89
Density	−0.77	−0.82	−0.76	−0.81	−0.88	—	−0.90	−0.87	−0.86	−0.83	−0.86
4C fat mass	0.78	0.84	0.80	0.80	0.89	−0.93	—	0.94	0.92	0.93	0.94
DXA fat mass	0.77	0.84	0.81	0.79	0.89	−0.88	0.98	—	0.95	0.97	0.98
DXA arm fat	0.81	0.87	0.80	0.79	0.91	−0.88	0.95	0.97	—	0.92	0.94
DXA leg fat	0.75	0.82	0.75	0.75	0.85	−0.86	0.95	0.97	0.93	—	0.93
DXA trunk fat	0.75	0.82	0.82	0.80	0.88	−0.87	0.96	0.98	0.95	0.93	—

[†]For Pearson's correlation coefficients, M values are above the diagonal, and F values are below diagonal. All correlations were significant at $P < 0.0001$. DXA, dual-energy X-ray absorptiometry; SDS, SD score; 4C, 4-component.

males ($P < 0.0001$, adjusted for age). The prevalence of obesity was 11.5% and 14.7% in males and females, respectively, and was uncorrelated with age. The numbers of subjects by pubertal stage 1–5 were 98, 60, 28, 24, and 50 males, respectively, and 87, 48, 34, 22, and 80 females, respectively; pubertal stage in 2 other subjects was not recorded.

LMS percentiles for 4C fat-free mass, 4C fat-free mass index, and 4C fat mass index, respectively, against age for each sex are shown in **Figure 2**. Fat-free mass increased with age in an S-shaped association in both sexes but reached substantially higher values in males. This sex difference was reduced but remained apparent when adjusted for height in the form of the fat-free mass index. Fat mass had no discernible curvilinear association with age, which was attributed in part to differing age-associations of individual fat depots as proxied by the 4 skinfold thicknesses (data not shown). *z* score and percentile reference data for each of fat-free mass, fat mass, fat-free mass index, and fat mass index by the 4C model for each sex are shown in **Tables 2–5**.

Correlation coefficients for adiposity SDS indexes by sex are shown in **Table 6**. All coefficients were ≥ 0.68 ($P < 0.0001$). Coefficients and SEs for intercepts and slopes for the regression of 4C fat mass SDS on each individual adiposity SDS value,

together with SEE values, are shown in **Table 7**. No intercept differed significantly from 0; however, most slopes were significantly < 1 , with the exception of DXA fat mass SDS in females. For DXA fat mass in males, the upper 95% CI of the slope was just < 1 (0.983). The smallest SEE values were obtained from DXA fat mass SDS (0.33 SDS in males; 0.21 SDS in females), whereas values for skinfold thicknesses were ~ 0.5 to ~ 0.6 SDS. Thus, in most cases, individual SDS underestimated 4C fat SDS in subjects with higher adiposity, and this effect was minimal for DXA whole-body data. Bland-Altman analysis of agreement between 4C and DXA values for fat mass SDS is illustrated in **Figure 3** and showed no systematic trend in bias across the range of adiposity but greater random inconsistency in subjects of low adiposity.

Correlation coefficients for SDSs for indexes of fat-free mass by sex are shown in **Table 8**. All correlations were ≥ 0.80 ($P < 0.0001$). Coefficients and SEs for intercepts and slopes for the regression of 4C fat-free mass SDS on each individual proxy SDS value, together with SEE values, are shown in **Table 9**. No intercept differed significantly from 0; however, most slopes were significantly < 1 , with the exception of DXA fat-free mass SDS in both sexes and TBW SDS in both sexes. SEE values were ~ 0.2 for DXA whole-body SDS, ~ 0.2 for TBW SDS, and

TABLE 7Intercepts, slopes, and SEEs for regression of 4C fat mass SDS on individual adiposity SDS[†]

Predictor	Males (<i>n</i> = 245)					Females (<i>n</i> = 259)				
	Intercept	SE	Slope	SE	SEE	Intercept	SE	Slope	SE	SEE
Biceps	0.044	0.036	0.840	0.036	0.55	0.032	0.040	0.802	0.041	0.63
Triceps	0.027	0.036	0.836	0.036	0.56	0.028	0.034	0.856	0.035	0.54
Subscapular	0.046	0.038	0.821	0.038	0.58	0.032	0.038	0.828	0.039	0.60
Suprailiac	0.037	0.037	0.862	0.038	0.58	0.037	0.039	0.820	0.039	0.61
Mean skinfold	0.038	0.026	0.837	0.026	0.40	0.028	0.027	0.826	0.027	0.41
Density	−0.017	0.028	−0.892	0.027	0.43	0.021	0.024	−0.927	0.024	0.38
DXA fat mass	0.018	0.021	0.941	0.021	0.33	0.010	0.013	0.979	0.013	0.21
DXA arm fat	0.002	0.025	0.926	0.025	0.39	0.017	0.018	0.946	0.018	0.30
DXA leg fat	0.006	0.024	0.931	0.024	0.37	−0.002	0.019	0.951	0.019	0.30
DXA trunk fat	0.010	0.023	0.923	0.022	0.35	−0.004	0.017	0.962	0.017	0.27

[†]The 4C fat mass SDS regressed on each individual adiposity SDS. All slopes were significantly different from 1 ($P < 0.05$) except for DXA fat mass in girls. DXA, dual-energy X-ray absorptiometry; SDS, SD score; 4C, 4-component.

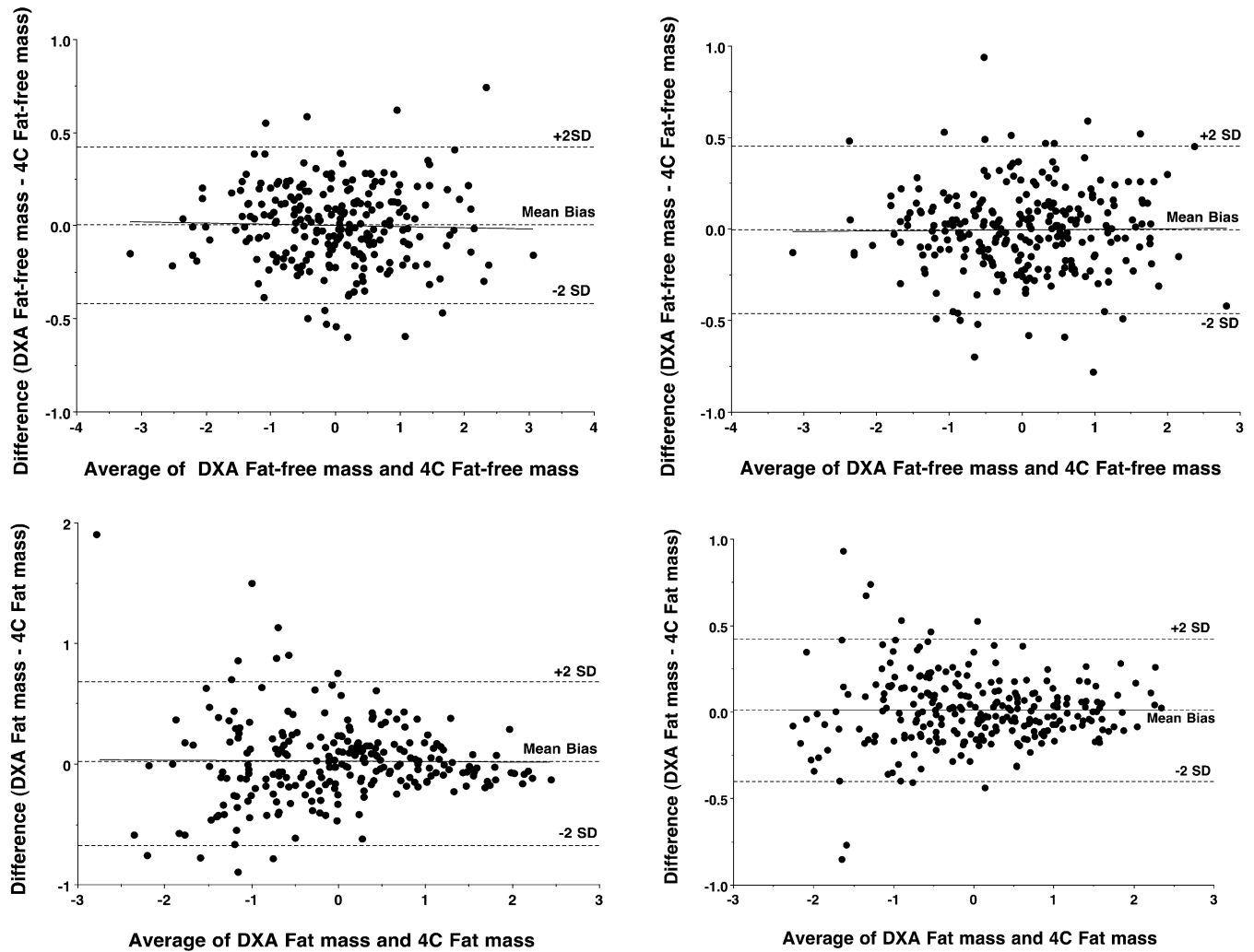


FIGURE 3. Bland-Altman plots illustrating the agreement between DXA fat-free mass SDS and 4C fat-free mass SDS (upper panels) and DXA fat SDS and 4C fat mass SDS (lower panels) in males ($n = 245$, left side) and females ($n = 259$, right side). The scatter plot shows agreement between techniques in individuals, and dotted lines show the mean bias and limits of agreement (± 2 SD of the bias). DXA, dual-energy X-ray absorptiometry; SDS, SD score; 4C, 4-component.

~ 0.44 for BIA SDS. The Bland-Altman analysis of agreement between 4C and DXA values for fat-free mass SDSs in males and females are illustrated in Figure 3 and showed no variability in bias across the range of fat-free mass SDSs.

r^2 values calculated from Tables 6 and 8 indicated that DXA fat SDS accounted for 88% and 96% of the variance in 4C fat SDS in males and females, respectively, whereas DXA fat-free SDS accounted for 96% and 94% of the variance in 4C fat-free SDS in males and females, respectively. In both sexes, agreement was better for fat-free SDS than for fat SDS (Figure 3), which showed poorer consistency between methods in subjects with low adiposity. For central fat, the correlation of DXA trunk fat SDS and waist SDS was 0.81 in males and 0.83 in females. Therefore, waist SDS explained 66% and 69% of the variance in trunk fat SDS in males and females, respectively.

Associations between 4C fat-free mass SDS and BIA SDS in each sex are shown in **Figure 4**. r^2 values from Table 8 indicated that height^2/Z SDS accounted for 83% and 81% of the variance in 4C fat-free SDS in males and females, respectively. The association between 4C fat mass SDS and the mean of 4 skinfold-

thickness SDSs in each sex is also shown in Figure 4. For each of the sum of 4 skinfold thicknesses, density, DXA indexes, and 4C fat mass, r^2 values calculated from Table 6 indicated that each individual adiposity SDS accounted for 66–96% and 61–96% of the variance in other SDSs in males and females, respectively.

DISCUSSION

Although reference data for children's body composition have long been desired, their development is complicated by the difficulty of obtaining accurate measurements. Advances in modeling, with the combination of several raw measurements, have allowed accurate 4C data to be obtained in children ≥ 4 y (31, 32). This approach is unlikely to be widely applied in clinical practice or research studies because of its expense and requirement for sophisticated equipment. Several techniques are used more routinely, including skinfold thicknesses, DXA, and BIA; however, each method uses different approaches to convert raw measurements to final body-composition values (37, 38).

TABLE 8Correlations for fat-free mass outcomes expressed in SDS format¹

M (<i>n</i> = 245) and F (<i>n</i> = 259) ²	Total body water	4C FFM	DXA FFM	DXA arm FFM	DXA leg FFM	DXA trunk FFM	Height ² /Z
Total body water	—	0.98	0.95	0.84	0.92	0.91	0.90
4C FFM	0.99	—	0.98	0.86	0.95	0.94	0.91
DXA FFM	0.96	0.97	—	0.88	0.96	0.97	0.91
DXA arm FFM	0.86	0.88	0.89	—	0.83	0.80	0.86
DXA leg FFM	0.94	0.95	0.96	0.84	—	0.87	0.89
DXA trunk FFM	0.91	0.93	0.97	0.82	0.87	—	0.86
Height ² /Z	0.90	0.90	0.90	0.84	0.87	0.86	—

¹For Pearson's correlation coefficients, M values are above the diagonal, and F values are below diagonal. All correlations were significant at $P < 0.0001$. DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; SDS, SD score; 4C, 4-component.

²For bioelectrical impedance analysis, $n = 195$ M and 227 F.

Over the past 2 decades, various pediatric body-composition reference data have been reported, including skinfold-thickness data in Spain (19) and the United States (18); BIA data in the United States (20), Turkey (21) and Japan (22); and DXA data in Sweden (23), the Netherlands (24), and the United States (25, 39). These data represent an advance over BMI, which can assess nutritional status but not fat and fat-free masses or their regional distribution. However, because of the different theoretical assumptions and population variability in body size and nutritional status, these heterogeneous data sets cannot easily be compared. To our knowledge, no study has previously reported reference data for a wide range of outcomes, which would allow future studies to benefit from convergence on a common data set, regardless of which technique was used.

We have attempted to resolve this problem by developing reference charts and SDSs for both the accurate 4C model and a number of simpler techniques across the age range from 5 to 20 y. We have further described correlations between SDSs calculated by using the different techniques and have shown medium-to-high agreement in all cases. Thus, whether measurements are made by using skinfold-thickness calipers, DXA, BIA, densitometry, isotopes, or the 4C model, there is relatively good ranking consistency, although different techniques cannot be used interchangeably when individuals are monitored over time. These new data will aid both single assessments of children and longitudinal monitoring over time. They are suitable for use in conditions in which there is no acute perturbation of water distribution (edema).

From our clinical experience, children with specific diseases are often able to undergo only a subset of body-composition measurements. For example, many patients are too sick to undergo plethysmography or DXA but can have BIA or TBW measured at the bedside (40, 41). Some obese children are too large to be successfully scanned by DXA and are difficult to measure by using skinfold-thickness calipers but can undergo plethysmography (42). When hydration varies beyond the normal range, and when patients are able to undergo a wider range of measurements, the 4C model is ideal, as we have shown for obesity (29), acute lymphoblastic leukemia (30), and cystic fibrosis (43). Thus, our reference data should substantially increase the capacity of clinicians to acquire and interpret data in a wide range of diseases, which could contribute to a range of components of clinical management. For more general community studies of nutritional status, TBW is the most accurate field method (32, 44) and can be applied in combination with our published reference data for hydration (38).

With the comparison between techniques for adiposity, the highest correlations with 4C fat mass SDS were shown for whole-body DXA fat mass SDS, with coefficients of 0.98 in females and 0.94 in males and an SEE ~ 0.2 SDS. The next best technique was density SDS, whereas the individual skinfold-thickness performed slightly less well (correlations ranged from 0.78 to 0.84, with an SEE ~ 0.6 SDS), but the average of the 4 skinfold-thickness SDS values had a correlation very similar to that of density in both sexes and an SEE ~ 0.4 SDS. For 4C fat-free

TABLE 9Intercepts, slopes, and SEEs for regression of 4C fat-free mass SDS on individual fat-free mass SDS¹

Predictor	Males (<i>n</i> = 245) ²					Females (<i>n</i> = 259) ²				
	Intercept	SE	Slope	SE	SEE	Intercept	SE	Slope	SE	SEE
Total body water	0.009	0.013	0.985	0.013	0.20	−0.002	0.009	0.987	0.009	0.15
DXA FFM	0.002	0.013	0.973	0.013	0.21	−0.006	0.014	0.977	0.014	0.23
DXA arm FFM	0.003	0.033	0.864	0.032	0.51	−0.014	0.030	0.881	0.030	0.48
DXA leg FFM	0.000	0.021	0.947	0.021	0.33	−0.007	0.020	0.949	0.020	0.32
DXA trunk FFM	0.002	0.022	0.920	0.022	0.34	−0.004	0.023	0.933	0.023	0.37
Height ² /Z	−0.012	0.030	0.911	0.030	0.42	−0.012	0.029	0.901	0.029	0.44

¹The 4C fat-free mass SDS regressed on each individual fat-free mass SDS. All slopes were significantly different from 1 ($P < 0.05$), except for total body water and DXA fat-free mass in both sexes. DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; SDS, SD score; 4C, 4-component.

²For bioelectrical impedance analysis, $n = 195$ M and 227 F.

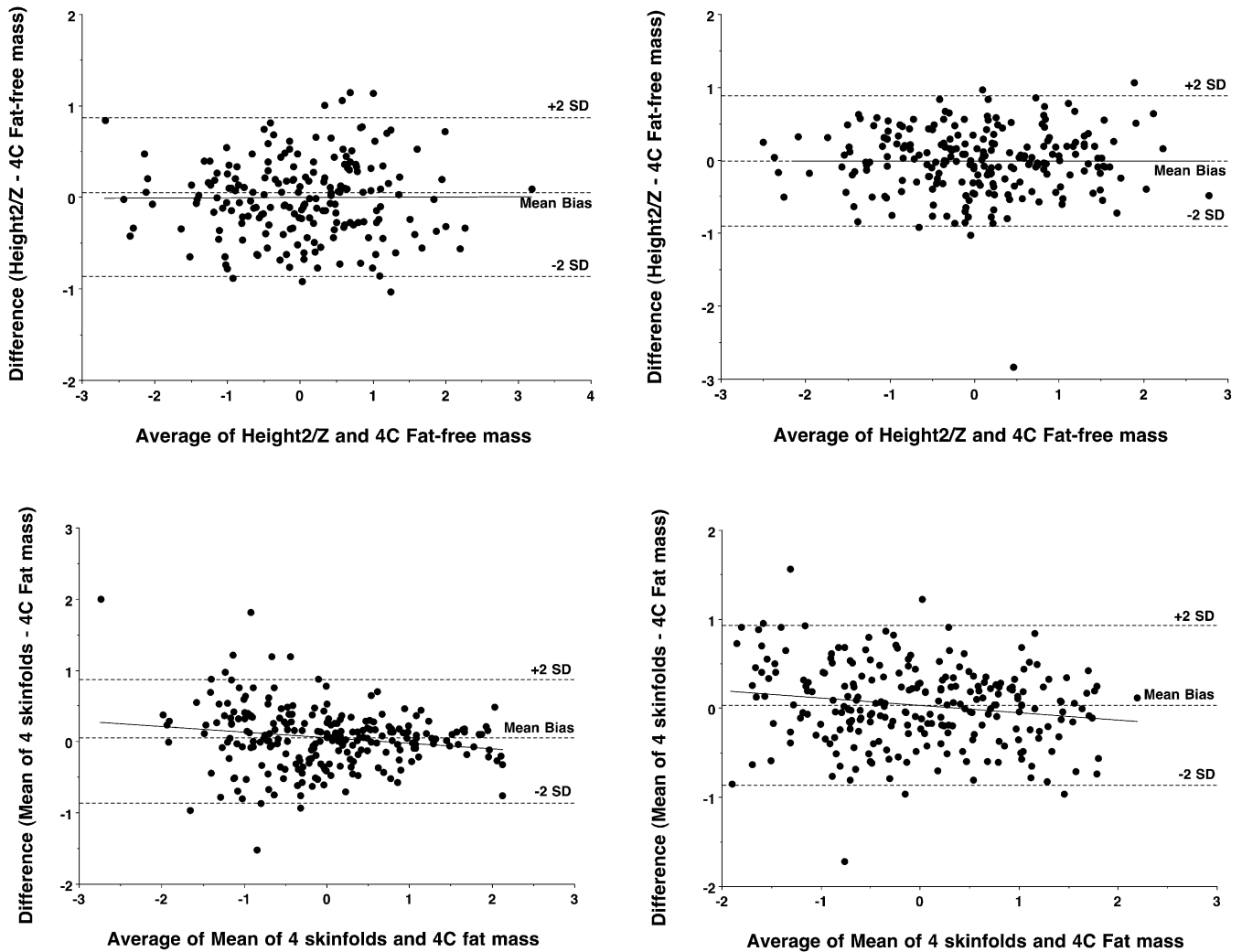


FIGURE 4. Bland-Altman plots illustrating agreement between height²/Z SDS and DXA fat-free mass SDS (upper panels) and the average of 4 skinfold-thickness SDSs and 4C fat mass SDSs (lower panels) in males ($n = 195$ for BIA and 245 for skinfold-thicknesses; left side) and females ($n = 227$ for BIA and 259 for skinfold-thicknesses; right side). The scatter plot shows agreement between techniques in individuals, and dotted lines show the mean bias and limits of agreement (± 2 SD of the bias). BIA, bioelectrical impedance analysis; DXA, dual-energy X-ray absorptiometry; SDS, SD score; 4C, 4-component.

mass SDS, DXA whole-body fat-free mass SDS likewise showed the highest correlations in both sexes of 0.98 in males and 0.97 in females and an SEE ~ 0.2 SDS. Other outcomes also showed high ranking consistency, with the least successful being DXA arm fat-free mass SDS (correlations of 0.86 in males and 0.88 in females and an SEE ~ 0.5 SDS). Therefore, for both primary outcomes, DXA whole-body SDS proved most consistent for the accurate ranking of individuals against the reference method, which explained 88–96% of the variance in 4C SDS values.

Therefore, although the absolute accuracy of DXA remains imperfect (42, 45, 46), its use for categorizing relative fat and fat-free masses on the basis of whole-body measurement appears the best simpler option if the 4C model is not available. Nevertheless, caution is required before extrapolation of our findings to other DXA instrumentation. Pediatric cross-calibration studies have shown relatively good agreement between different machines from a single manufacturer (47, 48) but poorer agreement between the machines of different manufacturers (49), and additional research is required before the use of other DXA instrumentation. Furthermore, for both DXA and other techniques, the consistency between 4C SDS and

other SDSs was poorer for adiposity at the lower end of the scale, especially in males, whereas for fat-free mass, techniques ranked with consistency across the whole range of the outcome (Figures 3 and 4). Thus, even DXA is a poor option compared with the 4C model when attempting to rank adiposity in leaner individuals.

Ideally, the interpretation of body-composition data requires adjustment for body size. This adjustment is particularly evident when children grow between 2 measurement occasions but is also important if a baseline assessment is made of patients who may have abnormal weight or height for their age. BMI represents the established index of weight adjusted for height in pediatric clinical practice. BMI may be divided into the following 2 components: the fat-free mass index and fat mass index. Each of these indexes is adjusted for height, and unlike the percentage of fat, the fat mass index is not confounded by variability in fat-free mass and, therefore, represents a more objective index of adiposity (17, 50). However, it has also been shown that, although fat-free mass scales with height², fat mass scales with height raised to a higher power (eg, height⁶ in 9 y-old children) (50). There is currently uncertainty over how best to adjust pediatric

body-composition data for size (51), and therefore, our new reference data for the 4C fat-free mass index and fat mass index represent a pragmatic preliminary attempt, which we intend to address further in future work.

A limitation of our study is that we were unable to extend the age range below 5 y. We have collected a large amount of isotope and skinfold-thickness data from 6 wk to 4 y (52); however, these data were collected a decade earlier than those reported in this article, and there is a poor statistical fit between the 2 data sets, most likely because of differential exposure to obesogenic environmental factors. Many patients who require body-composition assessment are aged <5 y; however, additional technical advances are required before our approach can be applied in this age range. A second limitation is that we were unable to include all possible techniques (eg, MRI and total-body electrical conductivity) or instrumentation. Our Lunar DXA data may not be appropriate for the instrumentation of other manufacturers, and our BIA data were collected by using standing instrumentation in combination with foot plates and hand grips and, hence, will not be entirely consistent with data collected from supine individuals by using adhesive electrodes. However, standing BIA removes a degree of interobserver error because it avoids the need to place electrodes on anatomical landmarks. A third limitation is that ethnic variability in our sample was not adequate to allow us to explore this issue in our analysis.

In conclusion, building on the pioneering work of Fomon and colleagues on their original reference child (53), we have described the measurements available in our new reference data set and provided examples of how the data can be presented; there are many alternative formats, and a large number of additional data are available from each of the 2-component techniques. We anticipate that the most appropriate use of the reference data will vary in clinical and research settings. To facilitate the use of the reference data by clinicians and researchers, we intend to make the data available through an Internet portal (<http://www.ucl.ac.uk/ich/research-ich/nutrition>), which will allow individual raw data for each technique to be entered with age and sex data to calculate SDSs (7). The graphs will also be available for download. Although for some purposes (eg, evaluation of cardiovascular risk), BMI SDS remains adequate for differentiating clinical status (54, 55), growth charts that allow the partitioning of weight into its fat and fat-free components are likely to be valuable for monitoring more-immediate effects of disease and responses to treatment (16).

The authors' responsibilities were as follows—JCKW, MSF, and TJC: designed the study; JEW, DH, SC, KK, and CG-E: collected raw data; JEW: modeled the data; CW: undertook all DXA scans; TD: undertook mass spectrometric analysis; JCKW: undertook LMS analyses under the supervision of TJC; JCKW: conducted statistical analyses and wrote the first draft of the manuscript; and all authors: contributed to revisions of the manuscript. None of the authors had a conflict of interest.

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